UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

EQB736 – BIOTECNOLOGIA EM COSMÉTICOS

Enzimas e Peptídeos em Cosméticos

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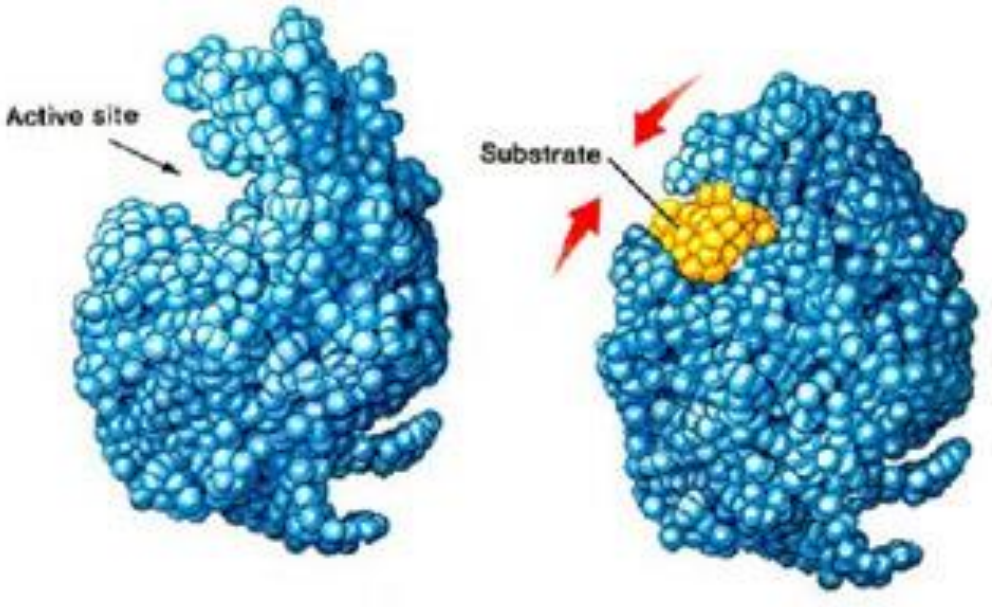
E-101/102/103, http://www.eq.ufrj.br/biose/

Enzimas

→ Biocatalisadores
→ Proteínas altamente especificas
→ Atuam eficientemente sob condições brandas, que são as requeridas para preservar a funcionalidade e integridade de sistemas biológicos



Classificação

ATP + D-Glicose ⟶ ADP + D-Glicose-6-fosfato

IUB - ATP:glicose fosfotransferase

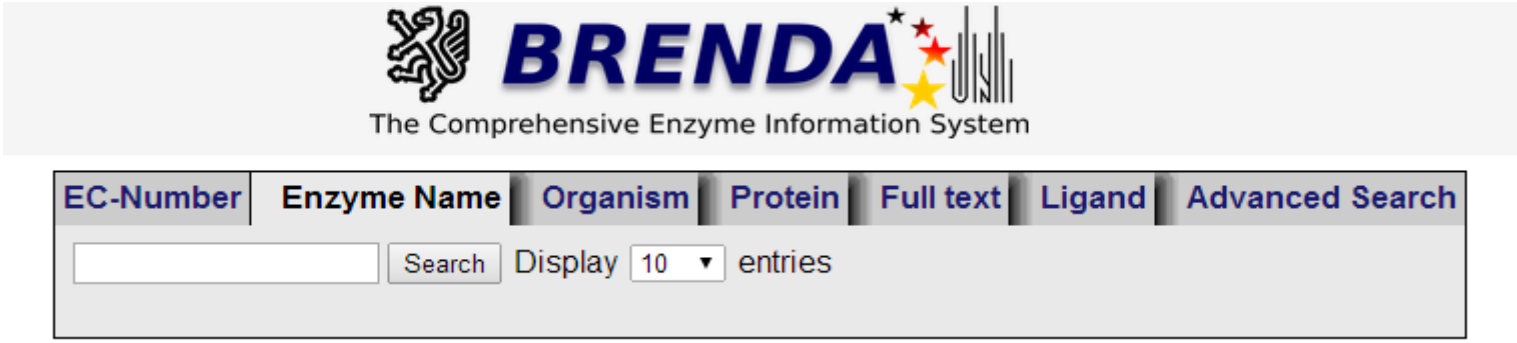
E.C. 2.7.1.1

2 - classe - Transferase
7 - subclasse - Fosfotransferases
1 - sub-subclasse - Fosfotransferase que utiliza grupo hidroxila como receptor
1 - indica ser a D-glicose o receptor do grupo fosfato

Nome trivial: Hexoquinase

Classificação

http://www.brenda-enzymes.org/



Nomenclature	Reaction & Specificity	Functional Parameters
Enzyme Names EC Number Common/ Recommended Name Systematic Name Synonyms CAS Registry Number	Pathway Catalysed Reaction Reaction Type Natural Substrates and Products Substrates and Products Substrates Natural Substrate Products Natural Product Inhibitors Cofactors Metals/Ions Activating Compounds Ligands Biochemicals Reactions Aligned	Km Value kcat/Km Value Ki Value IC50 Value pI Value Turnover Number Specific Activity pH Optimum pH Range Temperature Optimum Temperature Range **K**inetic **EN**zyme **DA**ta
Isolation & Preparation		**Organism-related information**
Purification Cloned Expression Renatured		Organism Source Tissue

Aplicações de Enzimas

- Detergentes
- Têxteis
- Papel e Celulose
- Biocombustíveis
- Cosméticos
- Síntese Orgânica
- Laticínios
- Panificação
- Suco
- Amido
- Cerveja

Mercado Mundial (2018): US$ 7,1 bilhões

Principais Empresas:

Novozymes, Dupont (Genencor, Danisco), DSM → 75% mercado

Quadro 16. Algumas enzimas envolvidas nos processos epidérmicos

Enzima	*Processo/ação*
Fosfolipase A_2	Conversão de fosfolipídios em ácidos graxos livres
β-glucocerebrosidase	Conversão de glucosilceramidas em ceramidas
SREBPs	Promove o aumento dos mRNA das enzimas envolvidas na síntese de ácidos graxos e colesterol de forma coordenada
Serina palmitoil transferase (SPT)	Catalisa a primeira etapa da síntese de esfingolipídios
Glucosilceramida sintase	Síntese de esfingolipídios
HMG-CoA redutase Farnisil fosfato sintase Esqualeno sintase	Enzimas da rota de síntese de colesterol
Esteroide sulfatase	Conversão de sulfato de colesterol a colesterol
Fosfolipases	Conversão dos diversos fosfolipídios oriundos dos CL a ácidos graxos livres, glicerolipídios e lisolecitina
Esfingomielinase	Conversão de esfingomielinas em ceramidas
Lipases neutras e ácidas	Conversão das acilglucosilceramidas a ceramidas
Ceramidase	Conversão de ceramidas a esfingomielinas
Reação não enzimática	Conversão não enzimática da glutamina a ácido pirrolidonocarboxílico (PCA) – umectante
Histidase	Conversão da histidina a ácido urocânico (absorvente UV)

Peeling

O termo *peeling* vem do verbo inglês *to peel* que significa descascar. Portanto, *peeling* pode ser traduzido como uma substância com a capacidade de acelerar o processo de renovação celular, agindo desde a camada mais superficial, camada córnea, até a camada mais profunda da derme, a camada reticular, com posterior reepitelização. A profundidade de um *peeling* irá depender de sua concentração, do pH e das características físico-químicas do ativo.

Conhecidos também como agentes ceratolíticos ou queratolíticos, as substâncias usadas nos *peelings* podem ser agentes químicos (ácidos) ou mecânicos (abrasivos). O uso de ácidos na estética é, sem dúvida, o procedimento que mais causa dúvidas e insegurança aos profissionais, e o receio de causar uma lesão na pele, devido ao efeito corrosivo do produto, faz com que poucos profissionais os utilizem. No entanto, aplicados com critérios, é um procedimento bastante seguro e eficaz, proporcionando inúmeros benefícios para a saúde e aparência da pele.

Quanto à profundidade[1,6,7]

1. ***Peeling* muito superficial** – Age apenas no estrato córneo.
2. ***Peeling* superficial** – Age na epiderme, até a camada granulosa ou basal.
3. ***Peeling* médio** – Age na derme papilar e só deve ser realizado por médico.
4. ***Peeling* profundo** – Age na derme reticular e também só deve ser realizado por médico.

Peeling

Quanto ao tipo

1. ***Peeling* físico ou mecânico** – Consiste no emprego de substâncias abrasivas para o arraste de células mortas. Age por atrito na camada córnea, através dessas substâncias que podem estar veiculadas em cremes, gel, gel-creme, ou até mesmo em loções. A aplicação consiste simplesmente em submeter à pele ao esfregaço, com massagens suaves e ligeira pressão.

Ex. Silica, sementes de frutas trituradas, microesferas de polietileno, argilas de feldspato, eletrotetrapia com microdermoabrasão com cristais de óxido de alumínio ("peeling de cristal")

***Peeling* químico** – Consiste na aplicação de substâncias químicas sintéticas ou vegetais, que geralmente são ácidos orgânicos (AHA), cujas concentrações podem variar, promovendo intensa renovação celular. A profundidade alcançada por essas substâncias pode variar, dependendo da concentração do ativo e de seu pH. Pode causar lesão da pele seguida de reepitelização.

***Peeling* vegetal (*gommage*)** – Consiste na aplicação de gomagem, uma espécie de látex, que, após um breve período de repouso, é manualmente atritado contra a pele, formando grumos. Durante a massagem, essa produto carreia as células mortas do estrato córneo, deixando a pele limpa e macia. Trata-se de um *peeling* leve, com o principal objetivo de limpar e melhorar a permeabilidade cutânea, facilitando assim a absorção de outros ativos aplicados em procedimentos subsequentes. Geralmente, o *peeling* vegetal não causa lesões, sendo o método ideal para peles sensíveis, nas quais o *peeling* biológico e o *peeling* químico seriam intoleráveis.

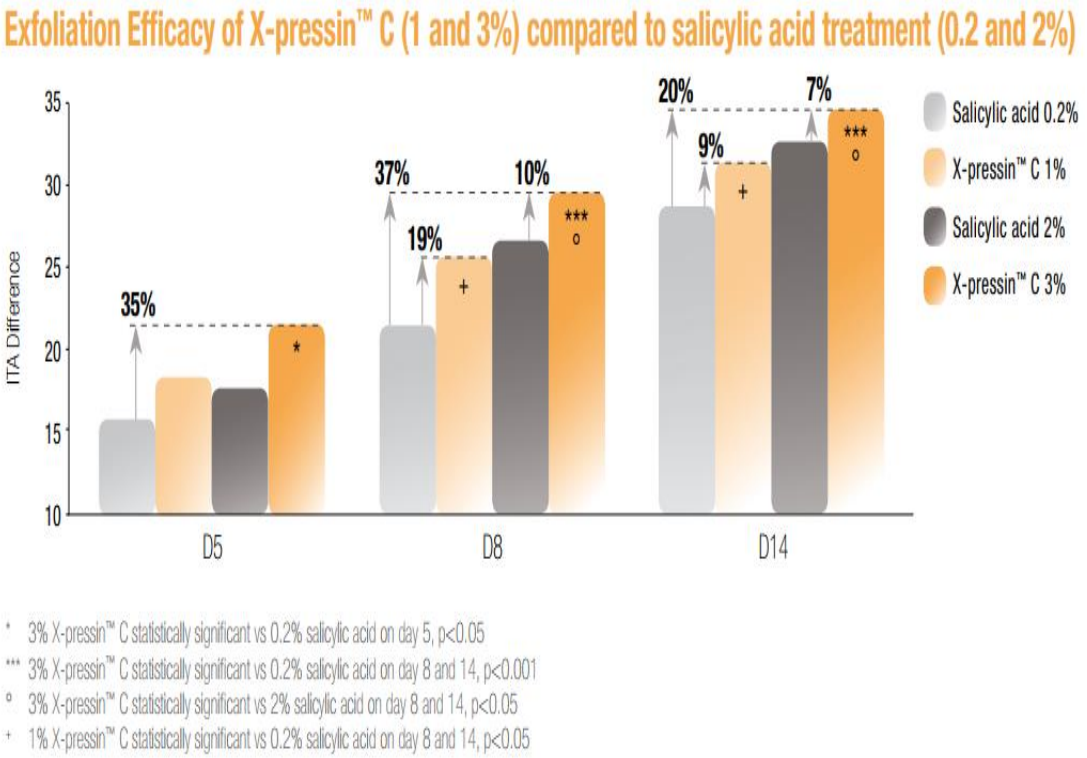
Peeling Enzimático

***Peeling* biológico** – Consiste no emprego de princípios ativos naturais com capacidade de promover a renovação celular através da hidrólise da ceratina. Essas substâncias são chamadas de enzimas proteolíticas e são capazes de romper as ligações entre os aminoácidos das proteínas. Os princípios ativos utilizados são enzimas biológicas que diminuem a espessura da camada córnea, dando à pele mais textura e plasticidade. Apesar de naturais, tais substâncias possuem caráter acentuadamente ácido, o que as torna tão eficazes quanto os *peelings* químicos. Devido ao seu caráter ácido, deve-se ter bastante cautela no uso desses produtos, a fim de evitar acidentes desagradáveis. Também é de suma importância o controle do tempo de aplicação.

As enzimas mais utilizadas são a papaína, extraída do mamão, e a bromelina, extraída do abacaxi.

X-pressin™ C

BASF



Pós-*Peeling*

Quadro 15. Cascata de eventos na regeneração da barreira cutânea

Evento	*Tempo após a quebra da barreira*	*Mediador bioquímico*/ controle*	*Inibidores do processo*
Formação de novas camadas lamelares	30 minutos a 2 horas		Membrana impermeável
Secreção de corpos lamelares pré-formados das camadas superiores do estrato granular	15 minutos		• Membrana impermeável • Monensina, efeito sobre a exocitose de vesículas • Brefeldin A, bloqueio do movimento dos lipídios e proteínas recentemente preparados • do retículo endoplasmático para o aparelho de Golgi (afeta a produção dos corpos lamelares)
Reposição de corpos lamelares nas células do estrato granuloso	30 a 60 minutos (após 3 a 6 horas, a situação já está normalizada)		Membrana impermeável

Pós-*Peeling*

Evento	*Tempo após a quebra da barreira*	*Mediador bioquímico*/ controle*	*Inibidores do processo*
Sinalização para o aumento da síntese de colesterol e ácidos graxos		Proteínas ligantes do elemento regulador de esterol (SREBPs), que levam ao aumento dos níveis de mRNA de diversas enzimas dos processos	
Aumento da síntese de colesterol (diretamente proporcional à intensidade da lesão) nas camadas superiores da epiderme	Imediatamente após a perturbação da barreira, retornando ao normal quando a barreira está recomposta	HMG-CoA redutase* (principal; enzima determinante da velocidade do processo de colesterogênese), HMG-CoA sintase,* farnisil pirofosfato sintase* e esqualeno sintase*	Membrana impermeável
Aumento da síntese de ácidos graxos (camadas inferiores da epiderme)	Imediatamente após a perturbação da barreira, retornando ao normal quando a barreira está recomposta	Acetil Co-A carboxilase,* ácido graxo sintase*	Membrana impermeável
Aumento da síntese de esfingolipídios	6 a 7 horas após a quebra da barreira	Serina palmitoil transferase** (etapa inicial da síntese)	
Aumento da conversão de glucosilceramidas a ceramidas		Ativação da β – glucocerebrosidase	Bromo-condritol--βepóxido, inibidor da enzima (composto do tipo conduritol)
Diminuição do cálcio no EG		• Liberação dos corpos lamelares • Aumento da síntese de profilagrina	

* Aumento de atividade e aumento nos níveis de mRNA das enzimas em processo.
** Aumento na atividade.

Inibidores de Tirosinase

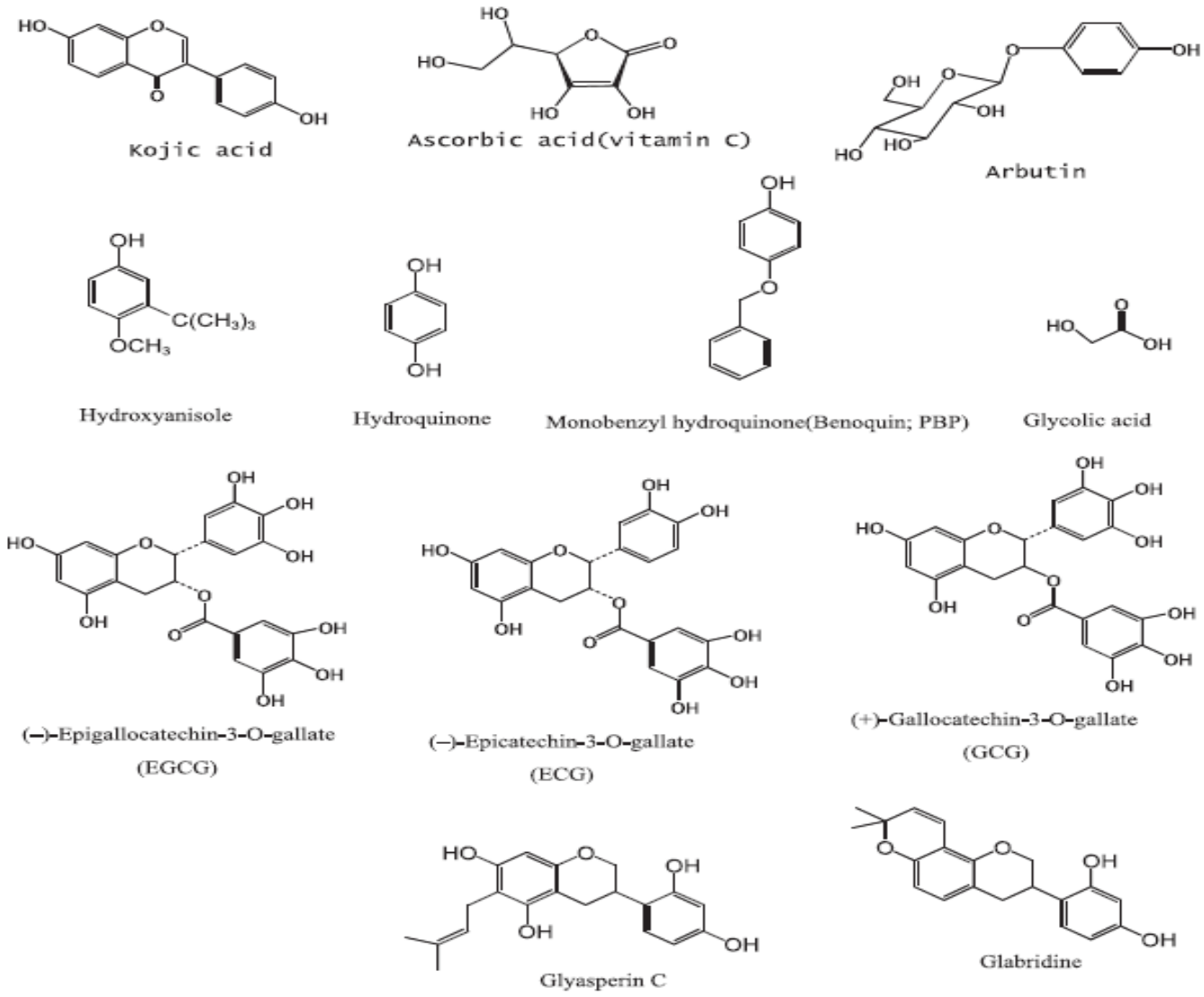


Figure 1. The chemical structures of known depigmenting agents.

Inibidores de Tirosinase

Commercial compounds found in mushrooms with reported cosmeceutical potential.

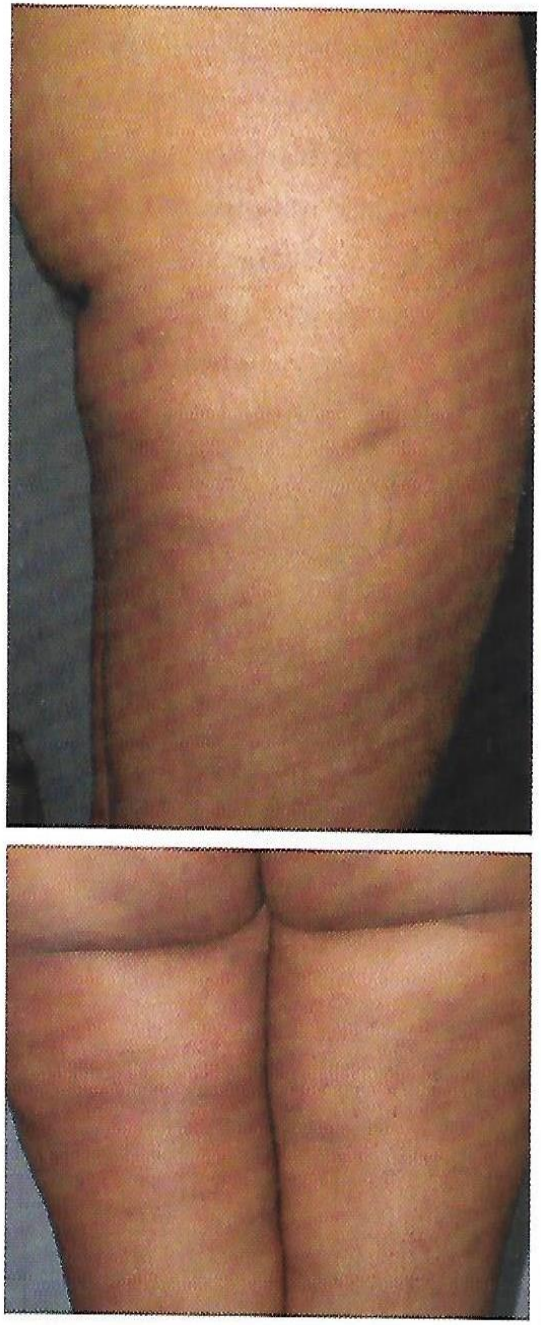
Compounds	Bioactivity	Effects
p-Coumaric acid	Anti-tyrosinase	At 10 μg/mL showed a higher tyrosinase activity inhibition than arbutin, but comparable to kojic acid
	Anti-tyrosinase	Reduced MITF and tyrosinase mRNA expression by 73 and 82%, respectively
	Anti-tyrosinase	Inhibited hyperpigmentation up to 77% in human skin
	Anti-collagenase	At 30 μg/mL inhibited MMP-1 expression from dermal fibroblasts
p-Coumaric acid, methyl *p*-coumarate	Anti-tyrosinase	Inhibited tyrosinase activity with an IC_{50} of 3 mM compared to methyl *p*-coumarate IC_{50} 30 mM
Ascorbyl-3-*p*-coumarate, Ascorbyl-2-*p*-coumarate	Anti-tyrosinase	At 100 μM, A-3-*p*-C and A-2-*p*-C decreased melanin content by 65 and 59%, respectively
Ascorbyl-3-*p*-coumarate, Ascorbyl-2-*p*-coumarate	Anti-collagenase	At 100–300 μM, A-3-*p*-C and A-2-*p*-C promote collagen release by 120–144% and 125–191%, respectively
Ergothioneine	Anti-collagenase	EGT at 2 mg/mL suppressed expression of MMP-1 protein by 52%
Dietary phenolic acids	Anti-tyrosinase	The IC_{30} (μM) values for the tyrosinase inhibition activity was *p*-coumaric acid 22.86 ± 2.1, Caffeic acid 43.09 ± 2.3 and Ferulic acid 51.85 ± 1.7
Dietary phenolic acids	Anti-tyrosinase	The IC_{30} (μM) values for the tyrosinase inhibition activity was caffeic acid 24.1 + 6.2 and ferulic acid >30 μM
Ellagic acid	Anti-collagenase	At 5 μM suppressed expression of MMP-1 and at 1–10 μM up regulated collagen levels
Gallic acid	Anti-tyrosinase	At 2.5–100 μM inhibited melanin content with an IC_{50} of 18.3 μM
Gallic acid	Anti-tyrosinase	At 0–400 μM reduces melanin synthesis via down-regulation of MITF
	Anti-tyrosinase	At 200 μM inhibited tyrosinase activity up to 85% and suppress melanin content in B16 melanoma cells
N-nicotinoyl tyramine (NNT)	Anti-tyrosinase	Suppressed expression of MITF and tyrosinase in a dose dependent manner
Hispolon	Anti-tyrosinase	At <2 μM inhibited the expression of tyrosinase and MITF up to 58.9–61.7%

MMP-1: matrix metalloproteinase-1; MITF: microphthalmia-associated transcription factor.

Celulite

Evolução da Lipodistrofia Ginoide

Grau	Aspectos clínicos	Identificação
I. Fase inicial	• Alterações metabólicas • Edema intersticial reversível	• Invisível a olho nu • Ausência de sensibilidade (dor)
II. Fase edematosa ou congestiva simples	• Extravasamento de exsudado • Estase circulatória venosa e linfática	• Manobras e pinçamento • Compressão da pele • Contração voluntária
III. Fase de polimerização	• Ruptura do equilíbrio hemodinâmico • Redução da elasticidade das fibras • Polimerização dos mucopolissacarídios • Aumento da viscosidade do meio • Maior retenção hídrica	• Irregularidades cutâneas visíveis pela mudança de posição • Alteração da sensibilidade (dor) • Pele com aspecto de casca de laranja
IV. Fase fibrosa ou esclerótica	• Aumento dos mucopolissacarídios • Aumento da fibrina • Diminuição do colágeno • Formação dos nódulos por hipertrofia dos adipócitos • Estrangulamento das estruturas vasculares e nervosas	• Visíveis em qualquer posição • Pele hipotônica (flacidez acentuada) • Sensibilidade aumentada • Aspecto de saco de nozes



Figuras 9.1 e **9.2** Lipodistrofia.

Celulite

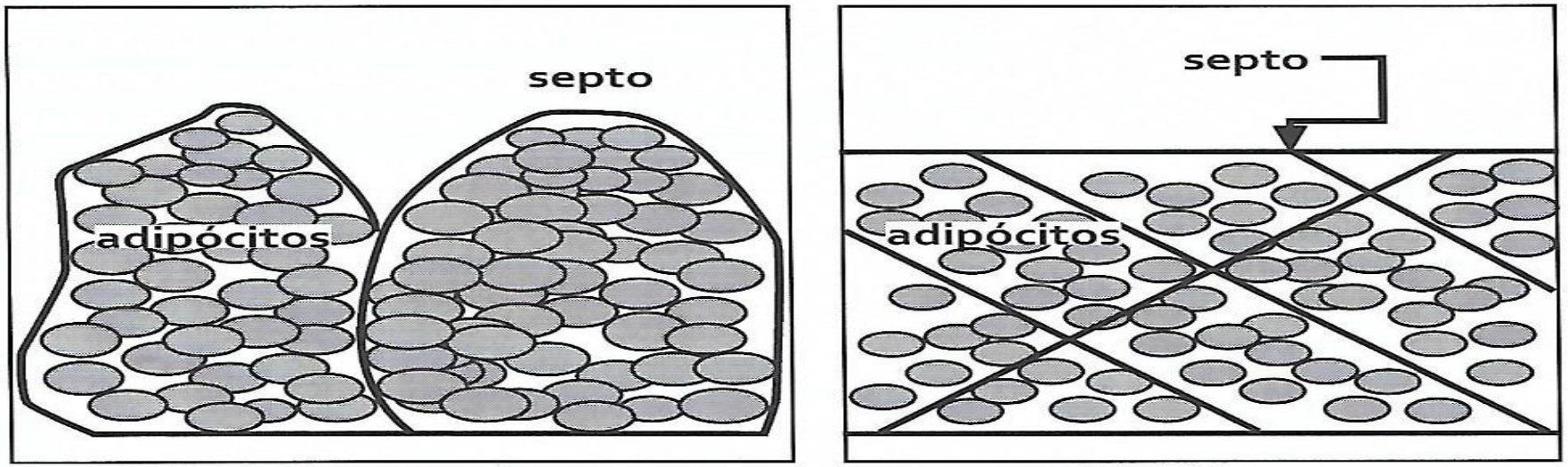


Figura 14.2 Estrutura esquemática do arranjo do tecido adiposo na mulher (à esquerda) e no homem (à direita). Nas mulheres, os septos têm disposição perpendicular. No homem, inclinam-se formando um ângulo de 45°.

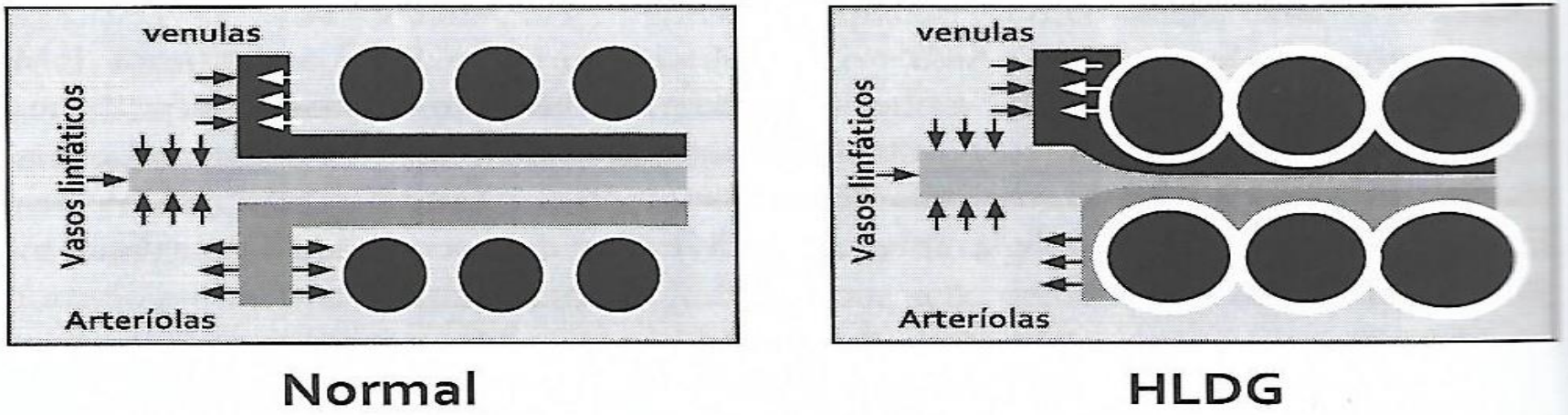


Figura 14.5 Representação esquemática do aumento da pressão dos adipócitos hipertrofiados sobre os vasos sanguíneos locais.

Celulite

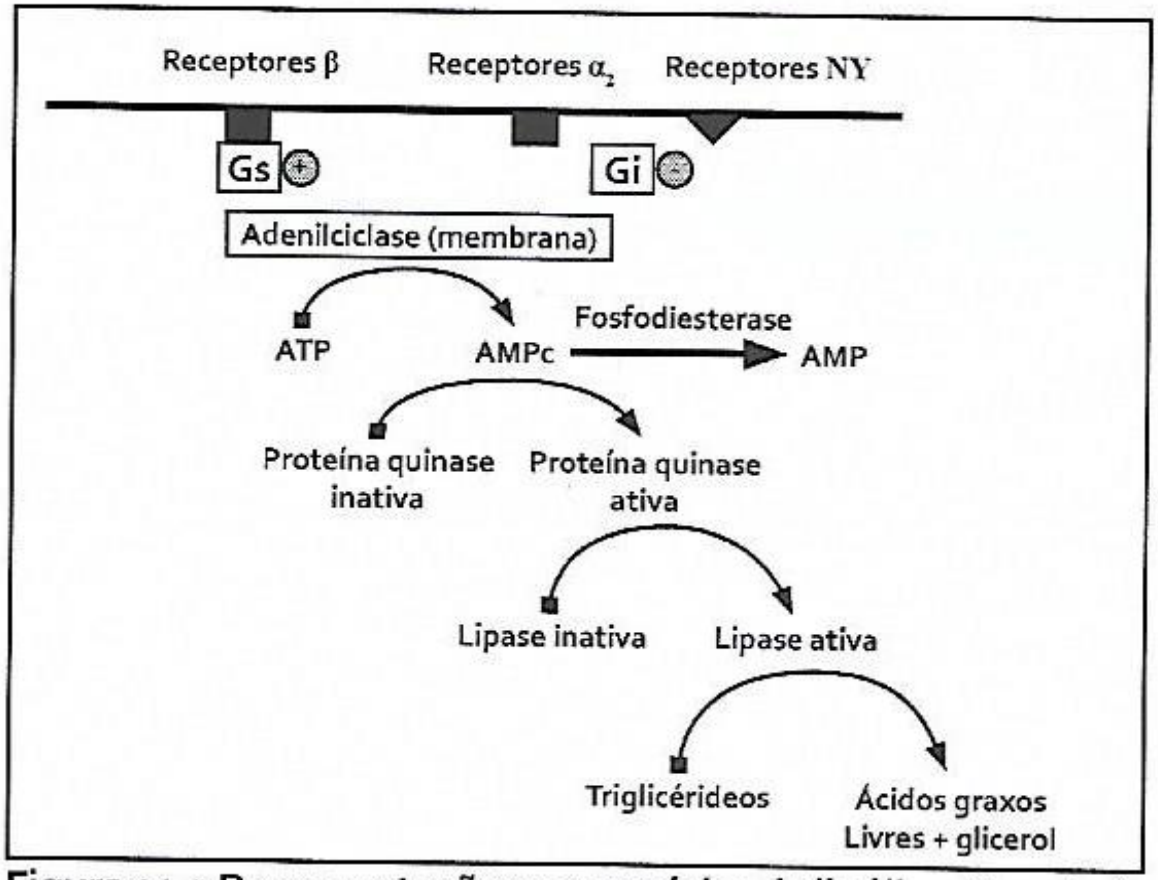


Figura 14.3 Representação esquemática da lipólise. Os adipócitos possuem em sua membrana receptores β-adrenérgicos que, uma vez estimulados, ativam a lipólise. Em contra-partida possuem receptores α2 e de neuropeptídeo Y (NPY) que, estimulados, inibem a lipólise.

Inibidores de Fosfodiesterase

Os mecanismos pelos quais um produto cosmético pode diminuir ou prevenir a HLDG são:[13]

- Diminuição da formação de triglicerídeos, ou seja, a lipogênese;
- Aumento da lipólise:
  - Inibindo a fosfadiesterase;
  - Bloqueando os receptores α_2;
  - Estimulando os receptores β;
  - Bloqueando os receptores neropeptídeos Y;
- Restabelecimento da microcirculação local;
- Diminuição do edema.

Inibidores de Fosfodiesterase

Cafeína, teofilina e aminofilina (Figura 14.6) possuem efeito lipolítico *in vitro*, atuando como inibidoras de fosfodiesterases (Figura 14.3). Permitem que a estimulação de receptores β adrenérgicos induzam a hidrólise de triglicerídeos com consequente liberação de ácidos graxos e glicerol.[7, 29]

Em formulações cosméticas, podem ser utilizadas as metilxantinas isoladas ou derivadas destas. Também podem ser utilizados os encapsulados de metilxantinas e extratos vegetais ricos em cafeína, como a noz-de-cola, guaraná e a erva-mate. Nestes casos, o teor de cafeína está diretamente ligado à qualidade do extrato.

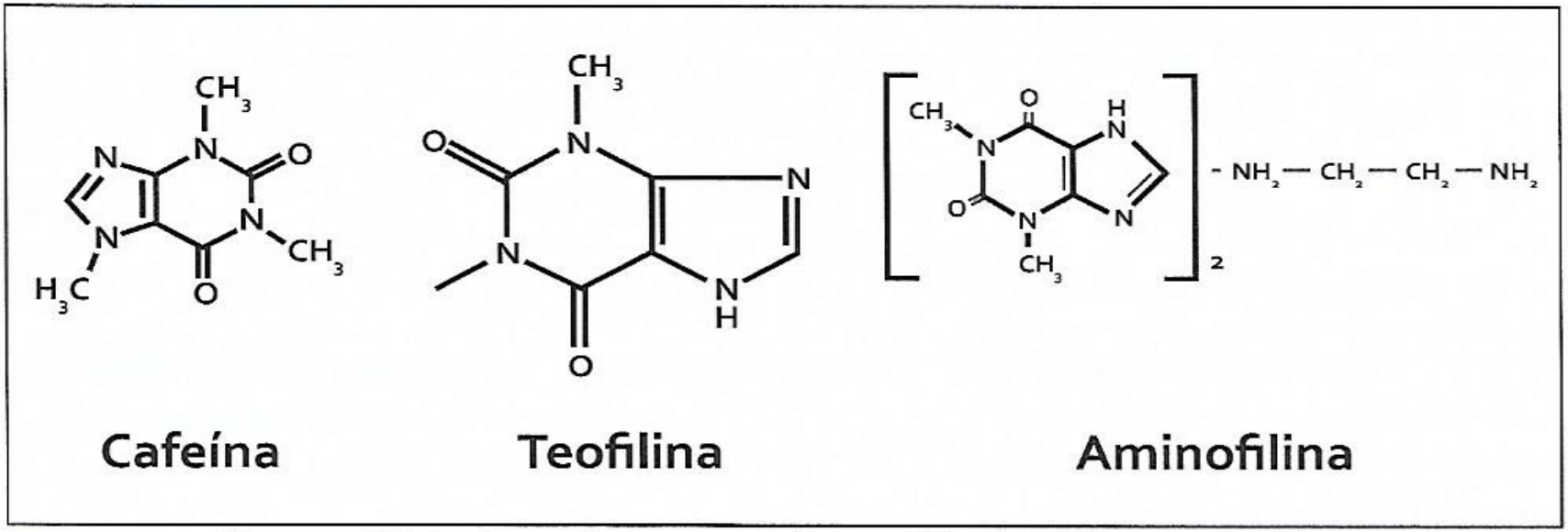


Figura 14.6 Estrutura química das xantinas: cafeína, teofilina e aminofilina.

Síntese de Ésteres

Overview of biocatalytic synthesis of carbohydrate fatty acid esters with surfactant properties.

Acyl acceptor	Acyl donor	Product	Enzyme	Solvent	Time (h)	Yield
Galactose	Oleic acid	Galactose oleate	Lipozyme RMIM (*Rhizomucor miehei*)	Ionic liquid and dimethylsulfoxide (DMSO)	2	87%
1,2-O-Isopropylidene-D-xylofuranose (IPXYL)	Arachidonic acid	Xylose-5-arachidonate	Lipase N-435 (*Candida antarctica*)	DMF	20	83–85%
Cellulose	Methyl methacrylate	Cellulose methacrylate	Novozyme 435 (*Candida antarctica*)	Ionic liquids (ILs)	72	89%
D-Glucose	Methyl methacrylate	D-Glucose methacrylate	Novozyme 435 (*Candida antarctica*)	Ionic liquids (ILs)	5	80%
Lactose	Oleic acid	Lactose ester of oleic acid	CALB-Chitosan CALB-Acrylic resin	Ethanol (99%)	72	84.1 ± 0.3% 83.6 ± 0.3%
Fructose	Oleic acid	Fructose ester of oleic acid	CALB-Acrylic resin CALB-Chitosan	Ethanol (99%)	72	74.3 ± 0.3% 70.1 ± 0.0%
Sucrose	Oleic acid	Sucrose ester of oleic acid	CALB-Acrylic resin CALB-Chitosan	Ethanol (99%)	72	56.1 ± 0.2% 55.0 ± 0.3%
D-(−) fructose	Oleic acid	Fructose oleate	Lipozyme (*Rhizomucor miehei*)	2-Methyl-2-butanol	26	83%
D-(−) fructose	Lauric acid	Fructose laurate	Novozym 435 (*Candida antarctica* B)	Ethyl-methylketone	72	82%
D-(−) fructose	Palmitic acid	Fructose palmitate	Novozym 435 (*Candida antarctica* B)	2-Methyl 2-butanol	72	78%
Sorbitol	Oleic acid	Mixture of sorbitol esters	*Chromobacterium viscosum* lipase	2-Pyrrolidone	74	80%
Xylitol	Capric acid	Xylitol caprate	Immobilized *Candida antarctica* lipase B	Solvent-free system	29	74.05%
α-Methylglucoside	Oleic acid	α-Methylglucosidedioleate	Lipase B from *Candida antarctica* (NS-40013)	Tert-butanol	40	72%
D-Fructose	Linoleic acid	Mixture of linoleic acid esters	NTG lipase (*Byssochlamys*	Tert-Butanol	24	71.30%

Síntese de Ésteres

Overview of biocatalytic synthesis of phenolic acid esters with antioxidant properties.

Acyl acceptor	Acyl donor	Product	Enzyme	Solvent	Time	Yield
2-Phenyl ethanol	Caffeic acid	Caffeic acid phenyl ester	(Novozym 435) from (*Candida antarctica*)	Isooctane	9.6 h	93.08 ± 0.42%
n-Butanol	Hydrocinnamic acid	*n*-Butyl hydrocinnamate	*Pseudomonas cepacia*	Hexane	48 h	92%
1-Propanol	Gallic acid	Propyl gallate	*Staphylococcus xylosus* lipase	Solvent free system	4 h	(90% ± 3.5)
2-Phenyl ethanol	Pure caffeic acid	Caffeic acid phenethyl ester	Novozym 435 (EC 3.1.13 *Candida antarctica* lipase immobilized on acrylic resin)	Isooctane	~48 h	>90%
Methanol	5-Caffeoylquinic acid	Methyl caffeate	*Cholgenate hydrolase*	1-Butyl-3-methylimidazolium bis (trifluoromethylsulfonyl)imide	–	–
3-cyclohexane-1-propanol	Methyl caffeate	3-Cyclohexylpropyl caffeate	*Cantarctica lipase B* (Novozyme 435)			85.3%
Arbutin	Ferulic acid	Feruloyl arbutin	B lipase from *Candida antarctica*	Tert-butanol	7 days	~62%
	α-Lipoic acid	Lipoyl arbutin				~90%
1-Octanol	Cinnamic acid	Octyl ester of cinnamic acid	Immobilized CaLB	Ionic liquids	72 h	56.2%
	Coumaric acid	Octyl ester of coumaric acid				27.4%
	Ferulic acid	Octyl ester of ferulic acid				17.5%
	Caffeic acid	Octyl ester of caffeic acid				8.4%
	Sinapic acid	Octyl ester of sinapic acid				0.4%
	p-Hydroxyphenyl acetic acid	Octyl ester of p-hydroxyphenyl acetic acid				62.6%
	p-Hydroxyphenyl propionic acid	Octyl ester of p-hydroxyphenyl propionic acid				60.6%
	3,4-Dihydroxyhydro cinnamic acid	Octyl ester of dihydroxyhydro cinnamic acid				35.5%
1-Octanol	Cinnamic acid	Cinnamic acid esters	*C. antarctica* lipase	Non-aqueous media	12 days	82%
			R. miehei lipase			59%
			C. rugosa lipase			19%
			F. oxysporum esterase			8%
			F. solani cutinase			12%
1-Octanol	p-Coumaric	p-Coumaric acid ester	*C. antarctica* lipase	Non-aqueous media	12 days	25%
			R. miehei lipase			22%
			C. rugosa lipase			16%
			F. oxysporum esterase			10%
			F. solani cutinase			10%

Síntese de Ésteres

Overview of biocatalytic synthesis of L-ascorbic esters with antioxidant properties.

Acyl acceptor	Acyl donor	Product	Enzyme	Solvent	Time	Yield
L-Ascorbic acid	Vinyl palmitate	Ester of ascorbic acid	Novozym 435	2-Methyl-2-butanol/ DMSO 95:5 (v/v)	8 h	90%
L-Ascorbic acid	Palmitic acid	Ester of ascorbic acid	Lipase from *Bacillus stearothermophilus*	Hexane	30 min	≤97%
L-Ascorbic acid	Vinyl esters (C8–C16)	Ester of ascorbic acid	Chirazyme L-2	T-butanol Ch	48 h	≤91%
L-Ascorbic acid	Methyl palmitate	Ascorbyl esters	Lipase B from yeast *Candida antartica*	2-Methyl-2-butanol	5 h	68%
L-Ascorbic acid	Saturated fatty acids	Ester of ascorbic acid	Novozym 435	2-Methyl-2-propanol	24 h	≤65%
L-Ascorbic acid	Saturated fatty acids (C10–C14)	Ester of ascorbic acid	Chirazyme L-2	Acetone	11 days	60 g/L
L-Ascorbic acid	Oleic acid	Ester of ascorbic acid	Novozym 435	2-Methyl-2-butanol	12 h	≤45%
L-Ascorbic acid	EPA and DHA ethyl esters	Ester of ascorbic acid	Novozym 435	2-Methyl-2-butanol	3 h	≥40%
Ascorbic acid	Palmitic acid	Ascorbyl palmitate	Candida antarctica lipase	Tert-butanol	2 h	~26%
L-Ascorbic acid	Palm oil	Palm oil based ascorbyl esters	Novozym 435	2-Methyl-2-butanol	16 h	70–75%

Síntese de Ésteres

Overview of biocatalytic synthesis of esters with fragrance and flavor properties.

Acyl acceptor	Acyl donor	Product	Enzyme	Solvent	Time	Yield
Butanol	Acetic acid	Butyl acetate	*Rhizopus oryzae*	Solvent free system Heptane Hexane	24 h	60% 80% 76%
Butanol	Butyric acid	Butyl butyrate	*Thermomyces lanuginosus* immobilized on styrene–divinylbenzene beads	*n*-Hexane	~5 h	14.5 mmol g^{-1} h
Cinnamyl alcohol	Vinyl acetate	Cinnamyl acetate	Novozym 435	Toluene	1 h	96%
Cinnamyl alcohol	Lauric acid	Cinnamyl laurate	Novozym 435	Toluene	2 h	60%
β-Citronellol	Lauric acid	Citronellol laurate	Novozym SP 435	Heptane	2 h	53%
Ethanol	Butyric acid	Ethyl butyrate	*Candida antarctica.*	*n*-Heptane	96 h	72.9%
Ethanol	Hexanoic acid	Ethyl hexanoate	*Rhizopus chinensis* CCTCC M201021	Heptane	72 h	96.5%
Geraniol	Ethyl acetate, Isopropyl acetate & Ethylenglycol diacetate	Geranyl acetates	*Fusarium oxysporum*	*n*-Hexane	96 h	80–90%
Ethanol	Valeric acid	Ethyl valerate	*Candida rugosa* lipase immobilized in microemulsion based organogels (MBGs)	Cyclohexane & Isooctane	9 days	>95%
Isoamyl alcohol	Acetic acid	Isoamyl acetate	Lipase from *Rhizomucor miehei*	*n*-Heptane	72 h	>95%
Isoamyl alcohol	Acetic anhydride	Isoamyl acetate	Novozym 435	*n*-Hexane	2 h	~100%
Isoamyl alcohol	Acetic acid	Isoamyl acetate	Lipase B from *Candida antarctica*	*n*-Hexane	36.5 s	35%
Isoamyl alcohol	Acetic acid	Isoamyl acetate	*Staphylococcus simulans* lipase	Solvent free system	8 h	64%
Isoamyl alcohol which was distillated from fusel oil	Acetic acid	Isoamyl acetate	Immobilized lipase from *Candida antarctica* (Novozym 435)	Solvent free system	4–8 h	75%
Isoamyl alcohol	Butyric acid	Isoamyl butyrate	*Rhizopus* sp. lipase	Solvent free system	48 h	75%
Isoamyl alcohol	Myristic acid	Isoamyl myristate	Novozym 435	Solvent free system	1 h	96%
L-Menthol	Butyric anhydride	L-Menthyl butyrate	*Candida rugosa* lipase	*n*-Hexane	8.7 h	72.6%
n-Amyl alcohol	Isobutyric acid	*n*-Amyl isobutyrate	*Candida rugosa* lipase immobilized into poly (N-isopropylacrylamide-co-itaconic acid) hydrogels	*n*-Hexane	48 h	>90%
α-Terpineol	Acetic anhydride	Terpinyl acetate	*Candida rugosa*	*n*-Hexane and super critical carbon dioxide	1.5 h	95.1%

Síntese de Ésteres

Overview of biocatalytic synthesis of esters with emollient properties.

Acyl acceptor	Acyl donor	Product	Enzyme	Solvent	Time	Yield
Oleyl alcohol	Oleic acid	Oleyl oleate	Immobilized lipase from *Candida antartica*	*n*-Hexadecane	5 min	>90%
Ethanol	Oleic acid	Ethyl oleate	*Rhizomucor miehei* immobilized by adsorption on to a hydrophobic support—Accurel EP700	Organic solvent	24 h	~57%
2-Ethyl hexanol	Palmitic acid	2-Ethylhexyl palmitate	Lipase from *Candida* sp. 99–125 immobilized on surfactant modified cotton membrane (130 IU/cm^2)	Petroleum ether	30 h	95%
Isopropyl alcohol	Palmitic acid	Isopropyl palmitate	Lipase Novozym 435 (from *Candida antarctica*)	Organic solvent	2 h	~87%
Cetyl alcohol	Oleic acid	Cetyl oleate	*Candida* sp. 99–125	Solvent free system	8 h	98%
Octyl, decyl and dodecyl alcohol	Pyroglutamic acid (pGlu)	Octyl, decyl and dodecyl esters of pyroglutamic acid	*Candida antarctica* lipase	Acetonitrile	24 h	65–70%
Lauryl alcohol	Palmitic acid	Lauryl palmitate	Immobilized lipase from *Candida antartica* (Novozym 435)	Hexane	10 min	>90
Glycerol	Oleic acid	Monoglyceride (MAG) Diglyceride (DAG)	Lipase *Candida* sp. 99–125	Solvent free system	8 h 4 h	49.6% 54.3%
1-Octanol	Oleic acid	Octyl oleate	Lipozyme RMIM (*Rhizomucor miehei*)	SC-CO_2	5 h	88%
α-Butylglucoside	Lactic acid	α-Butylglucoside lactate	*Candida antarctica* lipase	2-Methyl-2-butanol	30 h	>95%
Glycerol	Tuna oil	Monoacylglycerols (MAG) rich in polyunsaturated fatty acids (PUFA)	*Pseudomonas fluorescence* immobilized on Accurel EP-100 (IM-AK)	Tert-butyl methyl ether	24 h	MAG was 24.6%, but containing 56.0 wt% PUFA
1-Octanol	Dihydrostearic acid	Octyl ester of dihydrostearic acid	Novozym 435	Hexane	5 h	82.9 ± 1.3%
1-Octanol	Dihydrostearic acid	Octyl ester of dihydrostearic acid	Lipozyme IM	Hexane	5 h	81.9 ± 0.6%
1-Hexanol	Lauric acid	Hexyl laurate	*Rhizomucor miehei* (Lipozyme IM-77)	*n*-Hexane	74.8 min	~97%

Síntese de Ésteres

Table 4 Protease-catalyzed reactions

Product	Donor	Acceptor	Enzyme	Solvent system	Yield (time)	T (°C)
7-*O*-Vinyl adipoyl kojic acid	Kojic acid	Divinyl adipate	Bioprase from *Bacillus subtilis*	Dimethylformamide	25 % (7 days)	30
7-*O*-Hexanoyl/octanoyl/decanoyl kojic acid		Vinyl hexanoate/octanoate/decanoate			13–27 % (7 days)	
6-*O*-Undecylenoyl p-hydroxyphenyl β-D-glucopyranoside	Arbutin	Undecylenic acid vinyl ester	Bioprase from *Bacillus subtilis*	Dimethylformamide	62 % (7 days)	40
3″-*O*-Vinylsuccinyl or vinylsebacoyl-rutin	Rutin	Divinyl succinate/sebacate	Subtilisin from *Bacillus subtilis*	Pyridine	12.8/19.8 % (4 days)	50
Vinylsuccinyl/vinylglutaryl/vinyladipoyl/dinylnonanedioyl/vinylsebacoyl/vinyltridecanedioyl-troxerutin	Troxerutin	Divinyl succinate/glutarate/adipate/nonanedioate/sebacate/decanedioate	Subtilisin from *Bacillus subtilis*(-enzyme pre-irradiated)	Pyridine	10.6–33.10 % (4 h)	50
2-*O*-Lauroyl-sucrose	Sucrose	Vinyl laurate	Alkaline protease from *Bacillus pseudofirmus*	Dimethylformamide:pyridine	50–60 % (24 h)	45
6-O-Vinyladipoyl-D-glucose/-D-mannose/-D-galactose/-methyl D-galactoside	D-Glucose/D-mannose/D-galactose/α-methyl D-galactoside	Divinyl adipate	Alkaline protease from *Streptomyces* sp.	Dimethylformamide	49–74 % (7 days)	35

Superoxido Dismutase

Superoxide Dismutase (SOD). A protective enzyme, SOD safeguards almost all living organisms from the damage caused by the free-radical oxygen species. Free-radical oxygen species damage cells by attacking unsaturated fatty acids in the cell membrane. In combination with the enzyme catalase, SOD completely converts these free-radical oxygen species into two water molecules plus oxygen. To further the interest in using SOD in cosmeceutical products, it is known that SOD tissue levels decrease with aging. Formulating products to contain stable SOD can be challenging, and the enzyme is irritating to skin. Modified SOD ingredients are available to aid formulation efforts.

The enzymes catalase and SOD do not react in similar ways to long-term UV exposure. A thorough investigation on humans, carried out over winter and summer seasons, confirms this fact: catalase, easily destroyed by UVA light in summer, is more active in winter, whereas SOD is much more resilient.[32] This then leads to a possibly harmful potential buildup of hydrogen peroxide in the skin during the summer. The need for a balanced antioxidant enzyme system thus becomes apparent.

Two approaches are possible:

- Stimulate or protect the innate enzyme system, so that even under UV exposure, it retains its efficacy; and
- Supply the lacking enzymes by topical application, for instance within sunscreen, pre-sun or post-sun products.

Hoppe and colleagues[33] and Maes and coworkers[34] present examples of the first strategy. They show that molecules such as salicin in skin fibroblasts[33] and vitamin D derivatives or betulinic acid in keratinocytes[34] are able to stimulate the synthesis of heat shock proteins that protect the catalase against UV-induced degradation. These molecules could therefore be used advantageously in sunscreens as antiaging actives inasmuch as they induce protection of the human antiaging defense systems.

The second approach has a few limitations. Available enzymes such as SOD and catalase, which is extracted from yeast or other biotech sources, usually are not easy to stabilize in cosmetic formulas, to say the least, because of their sensitivity to heat, oxygen and UV light. Furthermore, SOD alone on the skin would lead, at least

Envelhecimento Cutâneo

Fatores intrínsecos
Hormônios e genes

(A) células epiteliais da região interna do braço de uma idosa.

Fatores extrínsecos
Radição solar, Fumo

(B) células epiteliais faciais de uma idosa

Produção de Radicais Livres:
Superóxido, Oxigenio Singlete, Hidroxila

Metabolismo de Elastina
- Indução de tropoelastina por mRNA
- Acúmulo de elastina

(fibras elásticas desorganizadas)

Ativação de MAP quinases:
JNK, ERK, p38 quinase

Oxidação de componentes celulares: Lipideos, DNA e proteínas

(mutações e morte celular)

Ativação de NF-κB
Estimulo de transcrição de genes pró-inflamatórios de citocina:
IL-1, TNF-α, IL-6 e IL-8

Neovascularização
Regulação de VEGF, TSP-1 e TSP-2

Ativação do AP-1
Perda de resposta do TGF-β

Inflamação

Metabolismo de Colágeno
- Redução da síntese do procolágeno tipo I e VII
- Desorganização e destruição do colágeno
- Inativação dos inibidores de metaloproteases
- Ativação e indução de síntese de metaloprotases (MMP-1, 3 e 9)

(ZOUBOLIS e MAKRANTONAKI, 2011)

Envelhecimento Cutâneo

Non smoking twin/ smoking twin

Envelhecimento Cutâneo

sun avoidance vs sun worship

Envelhecimento Cutâneo

Envelhecimento intrínseco ou cronologico

a natural consequence of physiological changes over time, but individual genetics are responsible for some interference. Currently, telomeres, small DNA sequences present at the ends of chromosomes, are considered as essential elements in the intrinsic aging process. These structures, when intact, tend to extend the life of cells. With aging, due to a continuous replication, a shortening of these structures occurs, which can be repaired by telomerase. The maintenance of telomeres by the action of telomerase would lock the aging process, but this action can lead to carcinogenesis.

Ethnicity → difference in pigmentation. High levels of melanin pigmentation protect from the cumulative effects of photoaging. Black skin is more compact and has a greater amount of lipids, also considered a factor that influences the increased resistance to aging. Asian subjects were observed to develop wrinkles later and to a lesser degree of intensity as compared to Caucasians.

Anatomic variations → thinner skin regions, aging becomes more evident. This is especially noted on the eyelids, the thinnest area of skin in the human body. There is also variability in both the composition and the distribution of lipids in the skin.

Hormonal changes → Estrogens influence the synthesis of collagen by fibroblasts, lead to the increased synthesis of hyaluronic acid, promote water retention, and increase the extracellular matrix. Conditions of hypoestrogenism, such as occur in the menopause, can have a deep effect on the skin, because it becomes thinner and less hydrated. The replacement of estrogens provides benefits in the skin rejuvenation of women during menopause.

Envelhecimento Cutâneo

Envelhecimento extrínseco ou Fotoenvelhecimento

is due to controllable factors and occurs at different degrees of intensity, due to solar exposure, smoking, and gravity, as well as other general lifestyle factors, such as diet, sleep, and overall health.

Ambient conditions. High temperatures lead to increased water evaporation, while low temperatures provide a hardening and reduced water loss through the same mechanism, even with abundant air moisture. The appropriate formation of structural proteins and lipids in the skin depends on the environmental temperature.

Drugs. Hypocholesterolemic agents can induce xeroderma and desquamation.

Smoking. It has been identified as an important contribution to facial wrinkles, even more than solar exposure. There is a parallel between the smoking load and the emergence and intensity of wrinkles. Smoking induces several harmful modifications: elastosis, telangiectasias, and decrease of blood flow in the capillary vessels, leading to the deprivation of nutrients in cutaneous tissues. Additionally, there is a reduction of collagen fibers and elastin in the dermis and in the lung, plus an increase of free radicals. There is also an increase of keratinocytic dysplasia and skin roughness. Hormone replacement therapy does not bring cutaneous benefits to patients who have been long-time smokers.

Sunlight exposure. Photo-exposure induces an avalanche of molecular and cellular changes that trigger a fast and dynamic disorder in the skin, unlike intrinsic changes, which occur slowly, producing a generalized atrophy and few structural changes until age 50. The effects of sunlight on the skin are deep and represent up to 90% of the visible aging of the face's skin, mainly in patients with light skin.

Table 1 Comparing photoaging to intrinsic aging[23]

Characteristics	Photoaging	Intrinsic aging
Overall		
Metabolic processes	Pronouncedly increased	Slowed down
Clinical appearance	Nodular, leathery, blotchy Coarse wrinkles, furrows	Smooth, unblemished Loss of elasticity, fine wrinkles
Skin color	Irregular pigmentation	Pigment diminishes to pallor
Skin surface marking	Markedly altered, often effaced	Maintains youthful geometric patterns
Onset	As early as late teens	Typically 50s-60s (women earlier than men)
Severity	Strongly associated to degree of pigmentation	Only slightly associated to degree of pigmentation
Epidermis		
Thickness	Acanthropic in early stages, atrophy in later stages	Thins with aging
Proliferation rate	Higher than normal	Lower than normal
Keratinocytes	Atopic and polarity loss, numerous dyskeratoses	Modest cellular irregularity
Dermo-epidermal junction	Extensive reduplication of lamina dense	Modest reduplication of lamina densa
Vitamin A content	Destroyed by the sun exposure	Plasma content of retinol increases
Dermis		
Elastin	Marked elastogenesis followed by massive degeneration, dense accumulation on fibers	Elastogenesis followed by elastolysis – 'moth-eaten fibers'
Elastin matrix	Massive increase in elastic fibers, replacing the collagenated dermal matrix	Gradual decline in production of dermal matrix, only modest increase in the number and thickness of elastic fibers in the reticular dermis
Lysosyme deposition on elastic fibers	Increased	Modest
Collagen production	Decrease in amounts of mature collagen	Mature collagen more stable in degradation
Grenz zone	Prominent	Absent
Microvasculature	Abnormal deposition of basement membrane-like material	Normal
Microcirculation	Vessels become dilated, deranged	Microvessels decrease; remaining vessels do not change
Inflammatory response	Pronounced inflammation, perivenular histiocytic-lymphocytic infiltrate	No inflammatory response observed

Envelhecimento Cutâneo

Table 2 Photoaging therapies categorized by type of treatment/prevention strategy and disease severity[35]

Primary	Secondary	Tertiary
Photoprotection	Photoprotection	Chemical peelings
	Retinoic acid	Microdermabrasion/ microcoblation
	Antioxidants	Laser
	Estrogens	Botulinum toxin
	Growth factors/ cytokines	Fillers

Inibidores de Metaloproteases

The matrix metalloproteinases are enzymes present in the skin and responsible for the degradation of macromolecules that form the skin extracellular matrix. The extracellular matrix plays an important role in the different biological functions of the skin, because it establishes the skin's three-dimensional integrity.

The enzymatic balance of matrix metalloproteinases is naturally controlled by the presence of tissue inhibitors of matrix metalloproteinase. Aging and environmental insults, such as UV light, perturb this equilibrium in favor of excessive activity of the enzyme. Such excessive activity results in a collapse of the meshwork in the extracellular matrix and contributes to the visible effects of UV damage: wrinkling, loss of elasticity and dilation of surface micro-capillary vessels.

Inibidores de Metaloproteases

The MMP physiologic activity is controlled by numerous phenomena, such as the ECM-cell interaction and cell stimulation by specific ligands.[6,7] There are three principal ways to control MMP activity:

- Regulation of gene transcription;
- Conversion of the latent proenzyme to an active enzyme;
- Inactivation of the enzyme in the presence of selective inhibitors.

Inibidores de Metaloproteases

Regulation of gene transcription: Stress conditions, such as thermal shock and exposure to UV light, are known to stimulate the MMP gene expression. Regulation at the transcription level is mediated through the promoter region linking the transcription factor AP-1. UV light stimulates the formation of AP-1 and is a strong stimulus of certain MMPs.[8]

The transcription regulation of MMP consists of many control factors. This phenomenon has proved to be extremely complex up to the point where inhibitory factors may become stimulators, depending on the existing molecular environment.

Inibidores de Metaloproteases

Conversion of proenzyme to enzyme: For the most part, MMPs are synthesized as latent proenzymes. They have in common a peptide linear structure (**Figure 1**). The amino-terminal portion contains the pro-peptide sequence where the cysteine residue is located. The catalytic or active domain containing the zinc atom necessary for enzyme activity is situated at the center.

In the latent configuration, the cysteine residue acts as a chelating agent blocking free zinc, thus impairing function. The median domain is bordered by a carboxy-terminal domain (also called hemopexin domain because of a sequence similarity with this protein). This domain has been identified in all MMPs, except MMP-7, and contains the recognition site of the substrate and the natural inhibitors. Therefore, in the latent form, the pro-peptide, the catalytic and the hemopexin domains co-exist and keep the MMP inactive (**Figure 1a**).

The pro-MMPs must be activated before being able to cleave their substrates. Enzyme activation involves destabilization of the pro-peptide domain followed by a complete cleavage due to an autocatalytic action (action whereby the MMP itself proceeds to the detachment of the pro-peptide) (**Figure 1b**). The result is an active MMP where the zinc atom is exposed and ready to intervene in the degradation of the ECM macromolecules.

Inibidores de Metaloproteases

Inactivation by inhibitors: MMP can be regulated by a number of effectors. The natural regulator is called a tissue inhibitor of metalloproteinase (TIMP).[9] TIMPs can inhibit the enzymatic activity of MMPs.

TIMPs are produced by cells from various organs, including skin.[10] They bind the MMPs in a 1:1 stoichiometry in order to form reversible non-covalent complexes. In this bi-molecular complex, the MMP is inactive. The production of TIMP-1 (selective inhibitor of the MMP-1) and TIMP-2 (selective inhibitor of the MMP-2) diminishes with age.[11] The loss of the natural inhibitor TIMP relocates the balance towards an accrued activity of the MMPs and an excessive degradation of the ECM macromolecules.

The degradation mechanism that transforms the ECM molecules exists because of the actions of one or many members of the MMP family of enzymes, whose job it is to break down proteins; on the other hand, the members of the TIMP family are used to reduce the breakdown of proteins.[12-14] A well-orchestrated action between the MMPs and the TIMPs is essential in order to maintain a controlled enzymatic action and to maintain the integrity of skin ECM.

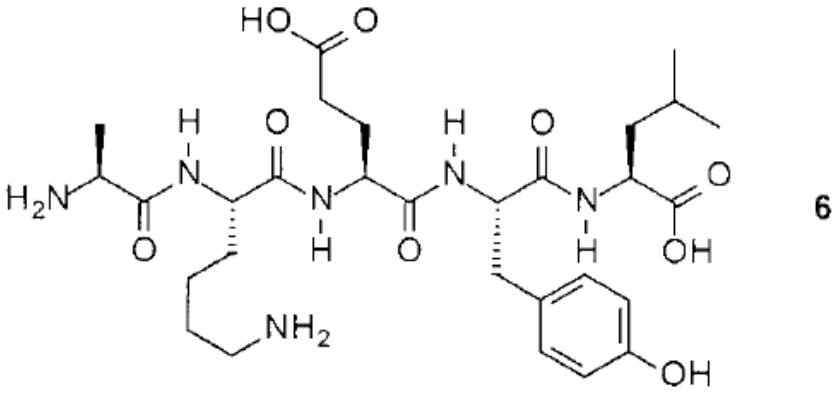
Inibidores de Metaloproteases

MMP inhibitors could contribute to the effectiveness of any cosmetic formulation that aims at:

- Delaying the appearance of fine lines and sagging skin;
- Reducing damage caused by exposure to the sun;
- Reducing skin redness;
- Reducing the appearance of spider veins (telangiectases);
- Reducing the appearance of dark circles around the eyes;
- Enhancing ECM cohesion;
- Improving the natural protective functions of the skin (against pollution, stress, age, sun); and
- Improving skin firmness and elasticity.

Peptideos

The nomenclature formally considers peptides as N-acyl amino acids.
“oligopeptides” are composed of fewer than 15 amino acids, “polypeptides” contain approximately 15–50 amino acids residues, and the expression “protein” is used for derivatives containing more than 50 amino acids.
Linear peptide sequences usually are written horizontally, starting with the amino terminus on the left side and the carboxy terminus on the right side.
Peptide bond is regularly formed between the C-1 (CO) of one amino acid and N-2 (N^{α}) of another amino acid.



6

Alanyl—lysyl—glutamyl—tyrosyl—leucine

H–Ala–Lys–Glu–Tyr–Leu–OH **6**

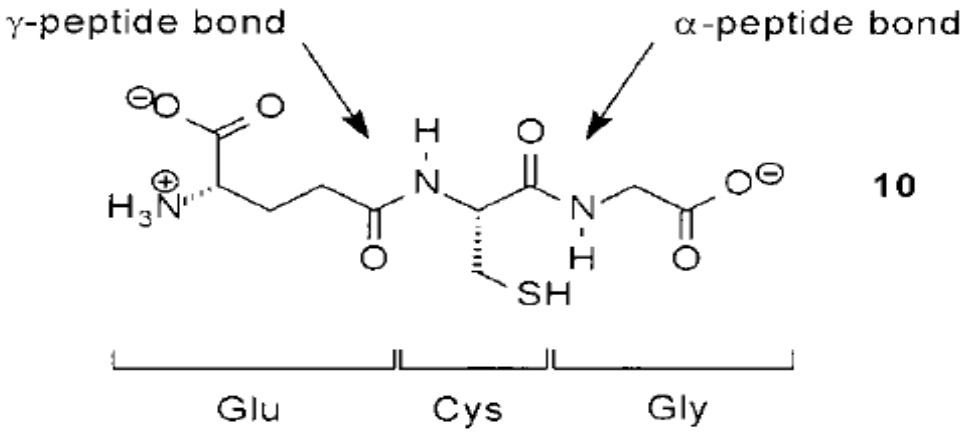
H–Ala–Lys–Glu–Tyr–Leu–$O^{\ominus}$ **7**

$\oplus H_2$–Ala–Lys–Glu–Tyr–Leu–$O^{\ominus}$ **8**

$\oplus H_2$–Ala–Lys–Glu–Tyr–Leu–OH **9**

Fig. 2.3 Structural formula, nomenclature, and three-letter code of the hypothetical pentapeptide iupaciubicin **6** in different ionic states (**7**–**9**).

Peptideos



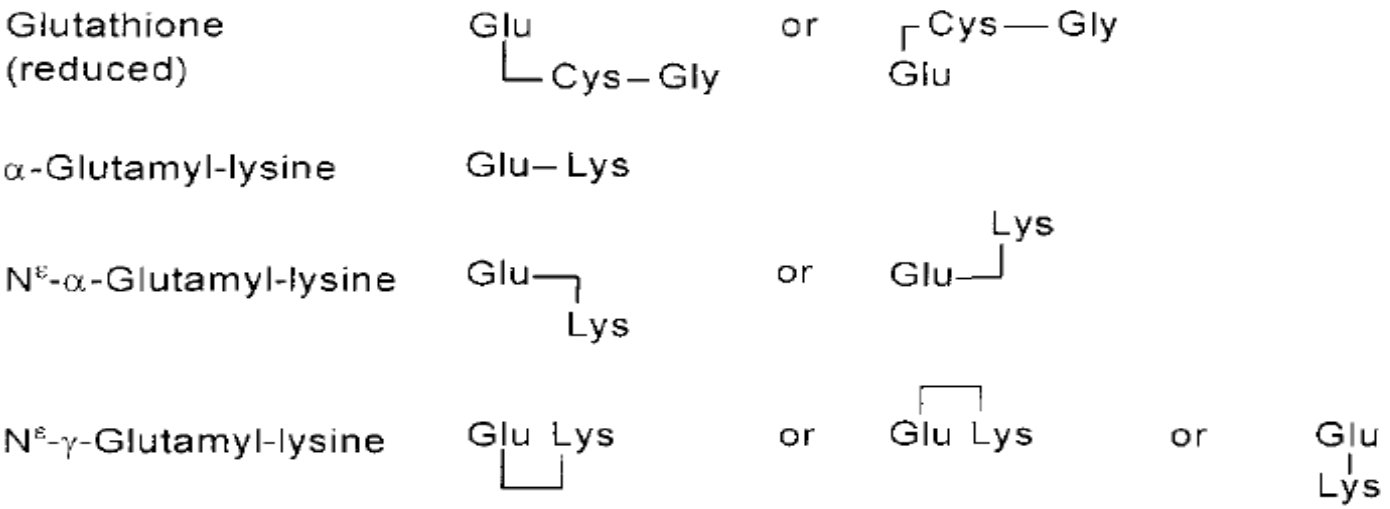


Fig. 2.4 Different connectivities of amino acids with additional side-chain functional groups lead to constitutional isomers, as exemplified for glutathione **10.**

Peptideos Bioativos

São cadeias curtas de aminoácidos com propriedades funcionais para alterar a fisiologia da pele, como a aumentar a produção de colageno, substituição da matrix extracellular, redução do tamanho e aparencia das rugas.

Uso de peptideos para uso topico é limitado pela natureza ionica dos aminoacidos, mas uma alternative seria sua esterificação com cadeia longas de ácidos graxos, como o palmitico, facilitando sua absorção pela pele.

Sua ação acosmética também vai depender de seu tamanho e sua permeabilidade membranar, permitindo seu uso em quantidades minimas, minimizando custo.

Tipos:

- Sinalizadores
- Carreadores
- Tensores
- Opioides
- Antioxidantes
- Antimicrobianos
- Surfactantes → lipopetideos

Peptideos Bioativos

Peptídeos sinalizadores → regulam a produção por fibroblastos de componentes da matriz extracelular. Subfragmentos de elastina e colageno poderiam agir como estimuladores retroativos induzindo sua propria sintese. Também podem diminuir a atividade da colagenase

- Oligopeptide-20, consists of 12 amino acids. This peptide increases collagen and hyaluronic acid in cultured keratinocytes and fibroblasts.
- Palmitoil Pentapeptideo-4 (Pal KTTKS) → aumenta a produção de colageno I e III pela estimulação de fibroblastos, e também produz fibronectina e elastina. Além disso, inibe a produção de glicosaminoglicanas na pele, o que normalmente é associado ao envelhecimento e danos por radiação solar. Pal KTTKS is a fragmente of procollagen I.
- Palmitoyl-lysine-threonine (pal-KT) → aumenta a diferenciação da epiderme, fibroblastos dermais e basement membrane zone. Within dermal fibroblasts, pal-KT increased collagen I, collagen IV, and fibronectin.

Peptideos Bioativos

Peptídeos sinalizadores naturais → Matrikines

Two classes of matrikines have been characterized: natural matrikines signal directly from the extracellular milieu, and cryptic matrikines (or matricryptins) require proteolytic processing to reveal the ligand or to release the ligand from its ECM protein. Matrikines and matricryptins are classes of ligand that have been characterized as subdomains of various ECM proteins capable of signaling to the cell through receptors, such as growth factor receptors. Members of the noncollagenous ECM proteins include laminins, fibronectins, and tenascins. Both tenascin-C and laminin-5 have epidermal growth factor (EGF) like repeats. Select EGF-like repeats of tenascin-C elicit mitogenesis and epidermal growth factor receptor (EGFR) autophosphorylation in an EGFR-dependent manner. The EGF-like repeats of laminin-5 also act as cryptic ligands revealed by matrix metalloproteinase-2 (MMP-2) degradation of the surrounding extracellular matrix.

Fibrillar collagens are synthesized as precursor molecules containing N-terminal and C-terminal propeptides that are cleaved off extracellularly by specific peptidases. It was identified the activities of small peptide fragments within procollagen I resulted in the identification of KTTKS, a subfragment within the carboxy-terminal propeptide (residues 197-241) capable of stimulating the production of both collagen and fibronectin. This peptide fragment dramatically augmented ECM production in subconfluent fibroblasts. It also stimulated type I and type III collagens and fibronectin production in a dose and time-dependent manner with no effect on total protein synthesis or on the ratio of secreted proteins to cell-associated proteins.

Peptideos Bioativos

Peptídeos sinalizadores

Hexapeptide valgly-val-ala-pro-gly (VGVAPG) → elastin fragment with chemotactic properties. It attracts cells to wound sites and significantly stimulates fibroblast proliferation within human skin. It also decreases the expression of elastin. Conversely, this peptide has been found in another study to induce proteolytic and inflammatory damage by upregulation of MMP-1 and MMP-3.

Tripeptide-10 citrulline (T10-C) is a decorin-like molecule, a leucine-rich proteoglycan directly involved in matrix organization. By binding to the surface of collagen molecules, decorin regulates their interaction with other collagen molecules, stabilizing and orienting them, thus establishing a uniform tissue shape, increasing tensile strength of collagen and reduces its disruption. With age, lacks functional decorin within the skin, and, it is replaced with a truncated, nonfunctional fragment known as decorunt. T10-C contains the collagen-binding site sequences of decorin and, like decorin, is able to regulate collagen fibers. Unlike other peptides, which increase the quantity of collagen, it increases the quality of the collagen, enhancing uniformity and increasing cohesion. T10-C showed a decrease in collagen fiber diameter, similar to decorin, which led to increased skin suppleness and firmness.

Tetrapeptide arg-gly-asp-ser → is a fragment of fibronectin, enhancing ECM structure, and enhances cell and collagen cohesiveness.

Peptamide-6 phe-val-ala-pro-phe-pro (FVAPFP), is derived from the yeast *Saccharomyces,* similar sequence as yeast heat shock proteins. It is a firming peptide that works by upregulation of growth factors and increasing collagen synthesis. This peptide has been shown to improve skin elasticity and deformation response.

Peptideos Bioativos

Peptídeos sinalizadores naturais → Matrikines

Elastic fibers are an important component of the extracellular matrix and consist of two elements, the microfibrils and matrix elastin, providing elasticity and resilience to tissues that require the ability to deform repetitively and reversibly. Elastin has a unique repeating sequence in the hydrophobic region. VGVAPG1 is a hexapeptide repeated multiple times in human, bovine, and porcine elastin molecules. Elastase-mediated elastolysis liberates elastin fragments called elastokines, which display a wide range of biologic activities in a number of normal and transformed cells and are best known for their chemokine-like activities that are chemotactic for fibroblasts, macrophages, monocytes, and polymorphonuclear neutrophils. Chemotactic sites on the elastin molecule have been identified containing the XGXXPG motif. Such XGXXPG sites are also present in multiple copies among fibrillin-1, -2, and -3, fibronectin, laminin, and several tenascins and collagens.

The best studied elastin-peptide, VGVAPG, is known for its chemotactic activity and MMP up-regulation properties. Elastin-derived peptides stimulated the growth of human skin fibroblasts and accelerated angiogenesis in the chick chorioallantoic membrane in an *in vivo* model. This hexapeptide stimulates pseudotube formation from human vascular and microvascular, endothelial cells in the matrigel and collagen models, as well as cell migration in an *in vitro* wound healing assay. Elastin-derived peptide effects were attributed to up-regulation of pro-MT1-MMP and pro-MMP-2 expression and activation at both the messenger RNA (mRNA) and protein levels.

Peptideos Bioativos

Peptídeps Sinalizadores

Acetyl tetrapepide-9 and -11 (AcTP1 and AcTP2, respectively) increase skin thickness and firmness. AcTP1 increases collagen I and lumican synthesis. AcTP2 stimulates keratinocyte growth and syndecan-1 synthesis.

PKEK, a tetrapeptide of amino acids pro-lys-glu-lys, reduces pigmentation by reducing the expression of interleukin (IL) -6, IL-8, tumor necrosis factor-α, proopiomelanocorticotropin, α-melanocyte-stimulating hormone, and tyrosinase secondary to UVB upregulation of these genes. Skin pigmentation is thus decreased by reduction of UVB-induced proinflammatory reactions.

Sirtuin genes may also be altered through the application of peptides. A biopeptide developed from the yeast *Kluyveromyces* has been shown to stimulate sirtuins within human skin cells, specifically SIRT1. Sirtuins enhance cell longevity by allowing transcription to occur. This occurs through deacetylation of silenced genes. SIRT1 has also been shown to increase manganese superoxide dismutase, resulting in enhanced repair of oxidative stress. Sirtuins are decreased in aging skin and it can thus be hypothesized that increasing them will result in skin longevity. Topical application reduces wrinkles and pigmented spots, along with improving skin texture and hydration.

Peptideos Bioativos

Peptídeos Carreadores

Carrier peptides are necessary to stabilize and delivery essential metals for wound healing and several enzymatic processes. Copper, the most well-known metal involved with carrier peptides, is an essential cofactor for collagen stimulation. Moreover, carrier peptides increase levels of both MMP-2 and MMP-2 m-RNA as well as tissue inhibitors metalloproteinases (TIMP) 1 and 2 and, for these reasons, they allow dermal tissue remodeling. The *tripeptide* glycyl-L-histidyl-Llysine (GHK) is used as a copper vehicle. Copper peptide, as a cosmeceutical, is reported to improve skin firmness and texture, and to decrease fi ne lines and to improve hyperpigmentation. Specifically within fibroblasts, this complex increases synthesis of dermatan sulfate and heparin sulfate.

GHK-Cu was initially described as a growth factor for a variety of differentiated cells. It is a potent chemotactic agent for monocytes/macrophages and mast cells. It stimulates nerve tissue regeneration, angiogenesis in vivo, and stimulates the expression of different components of extracellular matrix both in vitro and in vivo. The copper peptide also enhances wound remodeling by modulating expression of MMPs.

It has been speculated that at least a proportion of circulating GHK may be derived from the ECM-binding protein SPARC (secreted protein, acidic, rich in cysteine). This protein is expressed by endothelial cells during development and tissue remodelling and yields the GHK sequence specifically, upon degradation by proteases such as elastase, stromelysin, trypsin, and subtilisin. These are generally present in situations of matrix turnover and may endow GHK with a matrikine-like role.

Peptideos Bioativos

Peptídeos Tensores

Neurotransmitter-modulating peptides currently used in cosmeceutical products were developed as topical mimics of botulinum neurotoxin. In this regard, synthetic peptides that emulate the amino acid sequence of the synaptic protein soluble N-ethylmaleimide sensitive factor attachment protein (SNAP)-25 were shown to be specific inhibitors of neurosecretion at micromolar concentrations. A 6-mer peptide (Ac-EEMQRR-NH2), derived from the N-terminal domain of SNAP-25 (aa 12-17), interferes with the assembly of the SNARE ternary complex and inhibits Ca2+-dependent catecholamine release from chromaffin cells. This hexapeptide (acetyl hexapeptide-3) was Argireline (Lipotec).

Waglerin-1 from the venom of Wagler's pit viper (Tropidolaemus wagleri) is a 22-amino acid peptide selective for the adult form of nicotinic acetylcholine receptors (nAChRs) and causes paralysis by competitively antagonizing muscle nAChRs. A synthetic peptide Tripeptide-3 (beta-Ala-Pro-Dab-NH-benzyl x 2 AcOH), that mimics the effect of Waglerin-1 known as SYN-AKE (Pentapharm, Basel, Switzerland) for reducing wrinkles by inhibiting muscle contractions. It was assumed that this peptide binds to the epsilon subunit of the muscular nAChR, which prevents binding of acetylcholine to the receptor.

Pentapeptide-3 (Vialox), has been developed to reduce wrinkles and lines. It exhibits curare-like activity, as it is a competitive antagonist at the acetylcholine postsynaptic membrane receptor. Consequently, this prevents sodium ion channels from opening, thereby inhibiting depolarization and muscle contraction. This product is effective in smoothing periorbital, forehead and nasolabial fold expression wrinkles and provides an immediate tightening effect to the skin. Its recommended concentration is 0.05–0.3%.

Peptideos Bioativos

Peptídeos Opioides

Leuphasyl, a pentapeptide of unpublished amino acid sequence, is a modified enkaphalin that couples to the enkaphalin receptor outside of nerve cells. Enkephalins are endogenous opioids that inhibit neuronal activity by binding G-protein coupled inhibitory receptors, leading to the release of G-protein subunits (alpha, beta and gamma) in the cell, which subsequently close calcium ion channels and open potassium channels. The influx of calcium is necessary for vesicle fusion in the release of acetylcholine from the neuron to the muscle. Thus, enkephalin activity inhibits acetylcholine release across the synapse to the muscle, preventing muscle contraction.
In vitro studies measuring the modulation of glutamate release in neuronal cell culture showed reduced neurotransmitter release. In vivo studies measuring antiwrinkle activity using skin topography analysis of silicon imprints revealed decreased wrinkle depth. Interestingly, Leuphasyl and Argireline showed a synergistic effect when both peptides were applied to skin in in vitro and in vivo studies.

Milk proteins are a rich source of opioid peptides, released from casein and whey protein. The largest group are b-casomorphins released from b-casein. They show the same amino terminal sequence (Tyr-Pro-Pro-Fen); their bioactivity is determined by the presence of tyrosine residues in the N-terminal position. **Opioid peptides act on the central nervous system, affect pain perception and behaviour**. These peptides also act locally in the gastrointestinal system, e.g. they extend the transit time of ingest through the digestive tract.

Table 1 Summary of bioactive peptides currently marketed for inclusion as active ingredients in skin care products

Company	Name	Activity	Premix products	Source
Atrium	Tripeptide-2	ECM stimulation via MMP-1 inhibition	ECM-protect	Undisclosed
Atrium	Tripeptide-1	ECM stimulation via growth factor	Kollaren	HGF
Atrium	Acetyl tetrapeptide-2	Reduce loss of thymic factors	Thymulen 4	Thymopoieten
Atrium	Acetylpeptide-1	Melanin increase via MSH regulation	Melitane	MSH agonist
Atrium	Nonapeptide-1	Tyrosinase activation inhibition	Melanostatine	MSH antagonist
Grant Indust.	Palmitoyl hexapeptide-6	Dermal repair	Matrix Rebuilder	Innate immunity
Grant Indust.	Oligopeptide-10	Dermal protection	InvisaSkin-64	Innate immunity
Lipotec	Tripeptide-1	Inhibits collagen glycation	Aldenine, Trylagen	Human serum
Lipotec	Tripeptide-10 citrulline	Collagen fibrillogenesis	Decorinyl, Trylagen	Decorin
Lipotec	Acetyl tetrapeptide-5	Edema reduction by ACE inhibition	Eyeseryl	Undisclosed
Lipotec	Pentapeptide-3	Botox-like via mimicing enkephalins	Leuphasyl	Undisclosed
Lipotec	Acetyl hexapeptide-3 (or -8)	Botox-like via SNARE inhibition	Argireline	SNAP-25
Lipotec	Acetyl octatapeptide-1	Botox-like via SNARE inhibition	SNAP-8	SNAP-25
Lipotec	Hexapeptide-10	Increases cell proliferation and laminin V	Serilesine	Laminin
Pentapharm	Palmitoyl tripeptide-5	Collagen synthesis via TGF-β	Syn-coll	Thrombospondin I
Pentapharm	Dipeptide diaminobutyroyl benzylamide diacetate	Botox-like via acetycholine receptor	Syn-ake	Waglerin 1
Pentapharm	Oligopeptide-20	MMP inhibitor via TIMP	Pepha-timp	TIMP-2
Pentapharm	Pentapeptide-3	Botox-like via acetycholine receptor	Vialox	Undisclosed
Procyte	Copper GHK/AHK	Wound healing	Brand example Neova	Human serum
Sederma	Dipeptide-2	Lymph drainage via ACE inhibition	Eyeliss	Rapeseed
Sederma	Palmitoyl oligopeptide	Collagen synthesis via signalling	Eyeliss, Matrixyl 3000	Human serum
Sederma	Palmitoyl tetrapeptide-7 (formally -3)	Elasticity via IL6 reduction	Matrixyl 3000, Rigin	IgG/matrikine
Sederma	Palmitoyl pentapeptide-3	Collagen stimulation via signalling	Matrixyl	Procollagen
Sederma	Palmitoyl oligopeptide	Retinoic acid-like activity	Biopeptide-CL	Collagen
Sederma	Palmitoyl oligopeptide	Increases collagen and HA	Biopeptide-EL	Elastin

ACE, angiotensin I-converting enzyme; *ECM,* extracellular matrix; *HA,* hyaluronic acid; *HGF,* hepatocyte growth factor; *IgG,* immunoglobulin G; *MMP,* matrix metalloproteinases; *MSH,* melanocyte-stimulating hormone; *SNARE*, soluble *N*-ethylmaleimide sensitive factor attachment receptor; *TGF*-β, transforming growth factor-β; *TIMP,* tissue inhibitor of MMP.

Companies: Atrium Biotechnologies (Quebec City, QC, Canada); Grant Industries (Elmwood, NJ, USA); Lipotec (Barcelona, Spain), Pentapharm (Basel, Switzerland); Procyte (Photomedix, Montgomeryville, PA, USA); Sederma (Le Perray en Yvelines, France)

Peptideos Bioativos

Peptides with antimicrobial activity

Using natural sources of antimicrobial compounds has enormous potential because they have characteristics such as low toxicity and high specificity. The mechanisms of these natural antimicrobial compounds can be better understood if we compare their modes of action against bacterial (unicellular) and animal (multicellular) cells. Bacterial cells have a layer rich in negatively charged phospholipids pointing toward the external environment, facilitating their interactions with peptides, most of which are positively charged. In contrast, animal cells are mainly composed of uncharged lipids in the outermost layer, and the negatively charged regions are pointed toward the cell interior (cytoplasm). Despite great diversity in their primary structures, most antimicrobial peptides are similar in that they are **short amino acid chains composed primarily of cationic and hydrophobic amino acids (**containing sequences rich in the amino acids Gly and Leu, and presence of Arg residue in the peptide sequence, increasing interactions with bacterial cell walls, due its cationic characteristic)**.**

The low MM of the peptide fractions, the resulting higher exposure of the amino acids and their charges, and the formation of small channels in the lipid bilayer are related to their antimicrobial activity. These features promote interactions between the peptide and the membrane.

Ex. Lactoferricin B, comprising residues from 17 to 41 in the sequence of milk lactoferrin; 92-amino acid ovotransferrin peptide, OTAP-92; cysteine-rich peptide obtained by hydrolysis of oysters with alcalase and bromelain; Peptide fractions f (181–207), f (175–207) and f (164–207) from the carboxylic end of αs2-casein; gelatin hydrolysate with Alcalase; whey hydrolyzed by pepsin;

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Peptideos Bioativos

Peptídeos antimicrobianos naturais

Although the skin was formerly considered an inactive physical protective barrier, now major function of the skin is to defend the body by rapidly mounting an innate immune response to injury and microbial insult. Resident and infiltrating cells in the skin synthesize and secrete small peptides that exhibit a wide range of bioactivities aimed at restoring barrier function and protecting the body until that function has been restored.

β-defensins → cysteine-rich peptides of 36-42 amino acids and are stabilized by three disulfide bonds. The three best-characterized human β-defensins—human β-defensin (hBD-1), hBD-2, and hBD-3—have been detected in human skin and cultured keratinocytes.

cathelicidin (hCAP-18/LL-37) → found at high concentrations in its unprocessed form (hCAP-18) in the granules of neutrophils and is processed upon degranulation and release.

Innate immunity peptides are multieffectors that are capable of recruiting and activating antigen-presenting cells and serve as early warning signals to activate innate and adaptive immune systems. Defensins and LL-37 are both known for their chemotactic role on various cell types and stimulation of cytokines. Endogenous LL-37 stimulates wound vascularization and reepithelialization of healing skin and angiogenesis in an animal model. The ability of this peptide to close wounds has partly been attributed to an induction of keratinocyte migration through transactivation of the EGFR.

Since the first observation that patients with atopic dermatitis are relatively deficient in cathelicidin and hBD-2 and demonstrate increased susceptibility to bacterial and viral superinfection of the involved skin. Restoration of innate peptides to normal levels via external application could provide significant benefit.

Peptideos Bioativos

Innate immunity

Palmitoyl hexapeptide-6, a peptide designed using an innate immunity peptide template (Grant Industries, Elmwood, NJ) stimulates fibroblast proliferation and scaffolding, collagen synthesis, and cell migration, and is currently marketed for inclusion in antiwrinkle skin care products.

Another example comes from granulysin-derived peptides that suppress cytokine release stimulated by *Propionibacterium acnes*. A synthetic peptide designed to bind lipotechoic acid, oligopeptide-10, has been developed for inclusion in anti-acne products (Grant Industries, NJ).

Peptideos Bioativos

Peptides with antioxidant activity

An antioxidant's ability to remove free radicals (superoxide and hydroxyls) is determined by various factors, including chemical reactivity, the rate of removal of the compound, the fate of the product of the antioxidant–radical reaction, interactions with other antioxidants, concentration and mobility in the environment and the compound's absorption, distribution, retention and metabolism.

Antioxidants are defined as substances that significantly slow or inhibit the oxidation of a substrate. Currently, some artificially synthesized antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertbutylhydroquinone (TBHQ), are used to prevent oxidative damage in foods and biosystems. However, these products are being used less due to their potential risks to human health, such as DNA damage and toxicity.

Some peptides with antioxidant activity occur naturally in food, such as **Glutathione** (γ-Glu-Cys-Gly) **and carnosine** (β-alanyl-L-histidine) present in muscle tissues. They can donate electrons, chelate metals and ions and inhibit lipid peroxidation. Similar to other biological activities, the antioxidant properties of peptides are related to their composition, structure and hydrophobicity. The presence of the amino acids Tyr, Trp, Met, Lys and Cys was reported to be an important factor in the antioxidant activities of the peptides, especially due to their ability to reduce Fe^{3+} to Fe^{2+} and to chelate Fe^{2+} and Cu^{2+} ions. Aromatic amino acids, such as Trp, Tyr, Phe have phenolic, indole and imidazole groups, respectively, which can act as proton donors to electron deficient radicals and efficiently scavenge them. The basic amino acid His has shown great potential in radical scavenging as a result of the chelating, lipid trapping and decomposition of the imidazole ring.

Peptideos Bioativos

Major methods for measuring antioxidant activities of peptides *in vitro* and their respective mechanisms.

Method	Mechanism	Reaction	Measurement
DPPH	DPPH capture	DPPH radical (2,2-diphenyl-1-picryl-hydrazyl) reacts with hydrogen-donating antioxidants, changing the color from violet to yellow.	Reduction in the absorbance at 517 nm
ORAC	Peroxyl radical capture	The peroxyl radical, generated from the breakdown of AAPH [2,2′-Azobis(2-amidinopropane) dihydrochloride] in the presence of atmospheric oxygen, reacts with a fluorescent indicator to produce a non-fluorescent product. In the presence of antioxidants, the fluorescence is maintained.	Reduction in fluorescence (excitation at 485 nm and emission at 520 nm)
FRAP	Iron reducing power	In the presence of electron-donating antioxidants, the Fe^{3+}-TPTZ [2,4,6-Tripyridyl-S-Triazine] complex is reduced to Fe^{2+}-TPTZ, changing the color from light blue to dark blue.	Increase in the absorbance at 593 nm
ABTS	ABTS capture	The radical ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) is stabilized in the presence of hydrogen-donating free radicals, changing the color from dark green to light green.	Reduction in the absorbance at 734 nm
Ability to chelate transition metals (Cu^{2+})	Chelation of Cu^{2+}	Complexation reaction of Cu^{2+} with pyrocatechol violet to generate a colored product. The presence of antioxidants decreases the formation of the Cu^{2+}-pyrocatechol complex, reducing the intensity of the color.	Reduction in the absorbance at 620 nm
Ability to chelate transition metals (Fe^{2+})	Chelation of Fe^{2+}	Complexation reaction of Fe^{2+} with ferrozine, generating a colored product. The presence of antioxidants decreases the formation if the Fe^{2+}-ferrozine complex, reducing color intensity.	Reduction in the absorbance at 562 nm
TBARS	Quantification of lipid peroxidation products	Reaction of thiobarbituric acid with hydroperoxide decomposition products. Malonaldehyde is the main compound quantified. Absorbance and antioxidant activity are inversely proportional.	Increase in the absorbance at 532 nm

Peptideos Bioativos

Major methods for measuring antioxidant activities of peptides *in vivo* and their respective mechanisms.

Method	Sample analyzed	Animals	Tissue/organ analyzed	Mechanism of action and principle underlying measurement
Superoxide dismutase (SOD)	Peptide isolate from hydrolyzed pig plasma	Male adult Wistar rats	Liver	SOD is an enzyme that catalyzes the dismutation of superoxide radicals into hydrogen and oxygen, thus playing an important role in protecting cells against reactive oxygen species
Catalase (CAT)	Peptide isolated from hydrolyzed fish protein	Albino male adult Wistar rats	Erythrocyte lysate (blood)	CAT is an enzyme that converts hydrogen peroxide into water and oxygen, thus having one of the major mechanisms for removing free radicals in the organism
Level of reduced glutathione (GSH)	Protein isolates from seeds from Syrian rue (*Peganum harmala*)	Albino male rats	Liver and blood plasma	GSH is an intracellular reducer that plays an important role for protecting cells from free radicals, peroxides and other toxic compounds
Glutathione-S-transferase (GST)	Peptide isolated from hydrolyzed mussel protein	Male adult rats	Liver	GST is an enzymatic complex in the cytosol that catalyzes the binding of reactive electrophilic molecules with glutathione, facilitating the metabolism and excretion of toxins and consequently reducing cell damage and DNA damage
Glutathione peroxidase (GPx)	Hydrolyzed corn gluten	Male and female Kunming mice	Liver and blood plasma	GPx is an enzyme that catalyzes the reaction between hydroperoxide and reduced glutathione leading to the formation of glutathione disulfite and the product of hydroperoxide reduction
Measurement of the level of malonaldehydes	Hydrolyzed fish protein (*Salaria basilisca*)	Male adult Wistar rats	Liver and blood plasma	Malanodialdehyde is an intermediate product for lipid peroxidation and thus can be used as an indicator for the presence of free radicals

Peptideos Bioativos

Lipopeptides

They are amphiphilic cyclic peptides composed of ten or seven amino acids which are bonded with beta-hydroxyacids. Fengycin has ten aminoacids, iturin and surfactin seven aminoacids respectively. Surfactin–A has L-leucine, surfactin-B has L-valine and surfactin-C has L-isoleucine at the amino acid position involved in lactone ring formation with the C14-15 beta-hydroxy fatty acid. The length of the fatty acid chains vary from C13 to C16 for surfactins, from C14 to C17 for iturins and from C14 to C18 for fengycins, giving different homologous compounds and isomers (n, iso, anteiso) for each lipopeptide.

Bacillus subtilis was first reported to produce surfactin a lipopeptide, which has been used for pharmaceutical purposes and in food applications, as well. Lipopeptides are produced also from *Pseudomonas* sp. and *Arthrobacte*r sp.. However, *B. subtilis* is the most appropriate economic source for the production of lipopeptides from starchy substrates, as potato process effluents and cassava wastewater.

The surface properties depend on the hydrophobic character of the alkyl chain incorporated in the aminoacids. Surfactin possesses excellent foaming properties compare with sodium dodecyl sulfate.

Lipopeptides have been used in anti-wrinkle cosmetics, or due to their deterging and emulsifying activity, showing excellent washability and low skin irritation. Lipopeptides possess antibacterial and antifungal activity and could be used for the treatment and prevention of microbial infections and for products preservation, besides inhibition of the adhesion of pathogenic organisms.

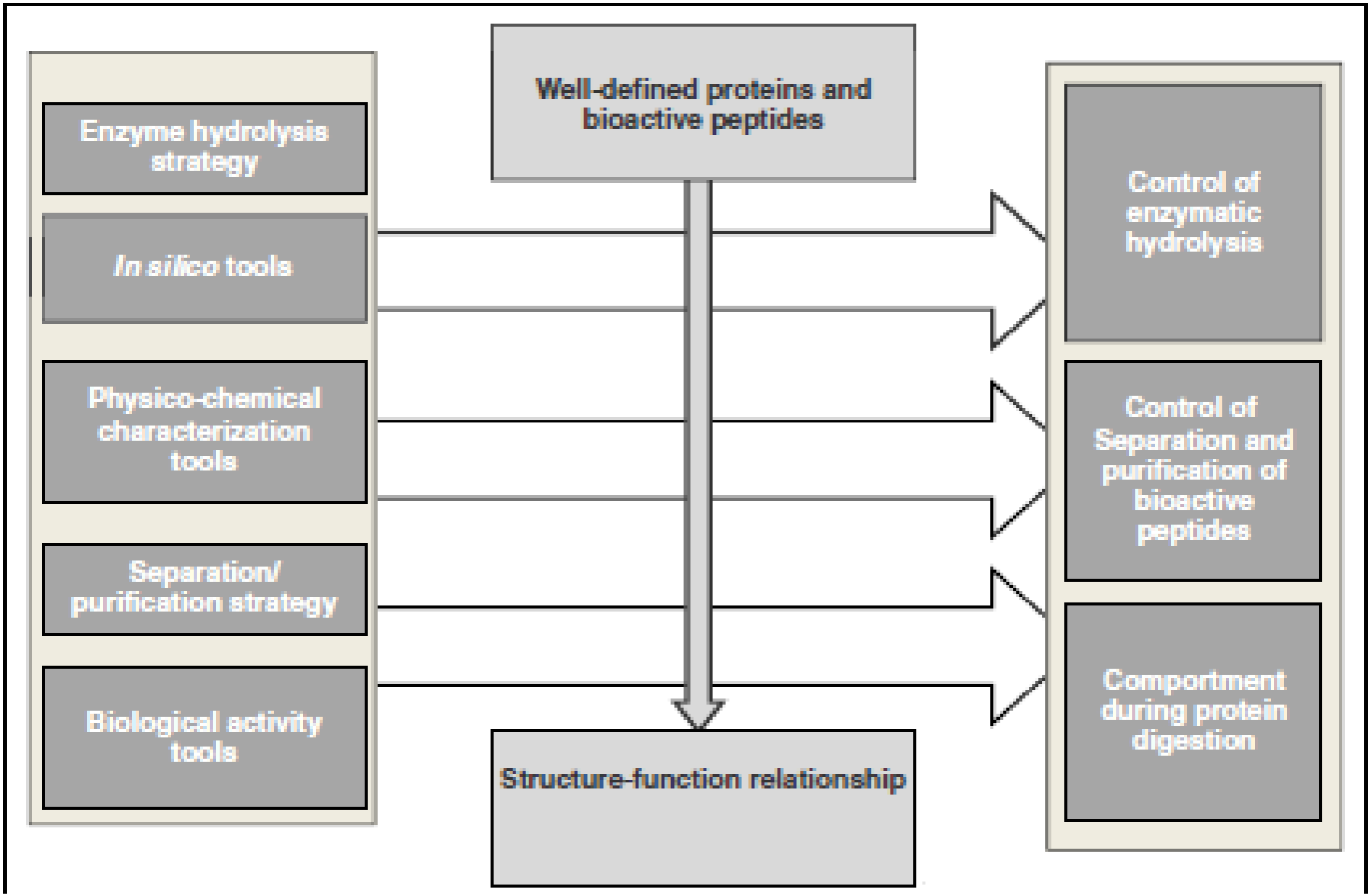
Peptideos Bioativos

Angiotensin-converting enzyme inhibition

A number of dipeptides and tripeptides have been identified as effective angiotensin-converting enzyme (ACE) inhibitors, and these have been isolated from a variety of natural sources. One of the most potent of such inhibitors is valine-tryptophan (VW), which has been shown to reduce blood pressure when delivered orally. ACE inhibitors act by preventing ACE from converting angiotensin I to angiotensin II, a potent vasoconstrictor, and by preventing the enzyme from inactivating the vasodilator bradykinin. The peptide VW has been marketed as dipeptide-2 (or Dipeptide VW) by Sederma. Under the intellectual property protecting this and related peptides for skin care use, it can only be used in combination with a second peptide and hesperidin, a compound that protects vasculature, is anti-inflammatory, and is an antioxidant. The premix is marketed in such a combination as Eyeliss, in which the second peptide is palmitoyl tetrapeptide-7.

The tetin class of peptides, first described in the 1990s, are fragments of immunoglobulins, interferons, ILs, or growth factors that modulate cytokine levels.89 A subgroup of this family, the rigins, derived from immunoglobulin G, also has been shown to down-regulate IL-6.90 One such peptide, palmitoyl tetrapeptide-7 (Pal-GQPR) has been developed as an active ingredient by Sederma and marketed as RIGIN. The ability of RIGIN to down-regulate IL-6 in resting and inflamed cells was compared with DHEA in vitro, and the two actives were comparable. Marketing materials related to RIGIN indicate that this reduction in IL-6 can produce increased skin firmness, smoothness, and elasticity.

Produção de Peptideos



Produção de Peptideos

Enzymatic hydrolysis (in vitro) from food proteins.

This procedure is preferred, especially in food and pharmaceutical industries → no residual organic solvents and toxic chemicals in the final peptide preparations.

This process is conducted under mild conditions which can be easily controlled and allows one to obtain products with well-defined feature. Most enzymatic modifications of dietary proteins are carried out by enzymes such as pepsin, bromelain, trypsin, chymotrypsin, papain or ficin under their respective optimal pH and temperature conditions. Also microorganisms are a relatively cheap source of proteases. For example, Neutrase, Subtilisin, Orientase, Protex 7L, Protamex 1.5 and proteases from lactic acid bacteria (LAB).

Proteins used: animal origin such as milk proteins (casein, whey protein), muscle proteins (myosin, collagen), delipidated egg yolk protein as well as of plant origin such as soy protein (glycinin, beta-conglycinin), wheat and rice protein, rapeseed protein (oil industry waste).

All proteases have a certain degree of specificity for the substrate, generally based on the sequence of amino acids directly surrounding the bond that is cleaved. This specificity and the hydrolysis conditions (pH, temperature, time) affect the size and the amino acid sequences in the peptide chains as well as the quantity of free amino acids, which can affect the biological activity of the hydrolysates.

Peptideos Bioativos

Using proteases to generate biologically active peptides from various protein sources.

Protease	Hydrolysis conditions	Protein source	Bioactivity of the peptides	Peptides	Identification methods
Alcalase™	pH 8.0; 50 °C; 3 h E:S = 1:20 [S] = 5.0%	Soy	Antiadipogenesis	Peptides with molecular weights between 754 and 3897 Da	Liquid chromatography mass spectrometry
Flavourzyme™	pH 7.0; 50 °C; 2 h E:S = 1:100 [S] = 2.5%	Purified soy protein	Antiadipogenesis	Peptides with molecular weights less than 1300 Da	High-performance molecular exclusion chromatography
Neutrase™	pH 6.0; 45 °C; 4 h E:S = 1:100 [S] = 2.5%	Purified soy protein	Antiadipogenesis	Peptides with molecular weights between 1300 and 2200 Da	High-performance molecular exclusion chromatography
Pepsin™	pH 5.5; 23 °C [S] = 1.0%	Bovine hemoglobin	Antimicrobial Antihypertensive	Peptides with molecular weights between 668 and 4430 Da	Electrospray ionization mass spectrometry (ESI/MS)
Alcalase™	pH 8.0; 50 °C; 3 h [E] = 0.2 mg/mL [S] = 8.0%	Bean	Antioxidant Anti-inflammatory	Peptides with molecular masses between 445 and 2148 Da	Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF)
Crude protease from *Bacillus licheniformis*	pH 10.0; 50 °C; 5.5 h [S] = 10.0%	Goby muscle	Anticoagulant	Leu-Cys-Arg His-Cys-Phe Cys-Leu-Cys-Arg Leu-Cys-Arg-Arg	Liquid chromatography Electrospray ionization mass spectrometry (ESI/MS)
Alcalase™ Flavourzyme™ Protamex™ Neutrase™ Pepsin Trypsin	pH 7.0; 50 °C; 8 h pH 7.0; 50 °C; 8 h pH 7.0; 50 °C; 8 h pH 7.0; 50 °C; 8 h pH 2.0; 37 °C; 8 h pH 8.0; 37 °C; 8 h	Salmon	Antioxidant Anti-inflammatory	Peptides with molecular masses between 1000 and 2000 Da	High-performance molecular exclusion chromatography
Crude protease from *Bacillus mojavensis*	pH 10.0; 50 °C [S] = 5.0%	Cuttlefish (*Sepia officinalis*) muscle	Antihypertensive	Peptides with molecular masses between 163 and 1047 Da	Liquid chromatography Electrospray ionization mass spectrometry (ESI/MS) Tandem mass spectrometry (ESI-MS/MS)

Produção de Peptideos

Fermentation

The proteolytic system of lactic acid bacteria (LAB) is complex and consists of three major components: proteases bound to the cell wall that promote the initial hydrolysis of milk casein into oligopeptides, specific transporters that transfer the oligopeptides to the cytoplasm and intracellular peptidases that finish the hydrolysis process to convert oligopeptides into free amino acids and/or low molecular weight peptides. The ability of these microorganisms to produce proteolytic enzymes makes them potential producers of bioactive peptides, which can be released during fermented product manufacturing. Ex. Lactobacillus helveticus, Lactobacillus delbrueckii ssp. bulgaricus, Lactococcus lactis ssp. diacetylactis, Lactococcus lactis ssp. cremoris and Streptococcus salivarius ssp. thermophylus. Although dairy products have been highlighted in scientific studies producing these peptides by fermentation, it has been shown that fermentation products derived from soy, beans, rice and wheat are also biologically active. Species of filamentous fungi such as Aspergillus oryzae and Aspergillus sojae have a long tradition of safe use in the production of fermented foods, in which several peptides with biological activities were detected, for example antioxidant and antihypertensive activities.

Peptideos Bioativos

Obtaining peptides with different biological activities by fermentation using various protein sources.

Microorganism	Protein source	Fermentation conditions	Peptides	Bioactivity
Streptococcus thermophiles *Lactobacillus bulgaricus* + *Protease Flavourzyme*	Soy milk	Submerged fermentation for 5 h at 43 °C	Tyr-Pro-Tyr-Tyr	Antihypertensive
Aspergillus oryzae	Rice, soy and casein	Solid-state fermentation for 40 h at 30 °C	Val-Pro-Pro; Ile-Pro-Pro	Antihypertensive
Aspergillus oryzae, *Rhizopus oligosporus*, *Actinomucor elegans*, *Bacillus subtilis*	Okara	Sequential submerged fermentation: *B. subtilis* for 48 h at 40 °C *A. oryzae*, *R. oligosporus* and *A. elegans* for 60 h at 30 °C	Not identified	Antioxidant
Aspergillus sojae	Soy and wheat	Solid-state fermentation for 192 h at 20–45 °C and 95% humidity	Gly-Tyr; Ala-Phe; Val-Pro; Ala-Ile; Val-Gly	Antihypertensive
Enterococcus faecalis TH563 *Lactobacillus delbrueckii* subsp. *bulgaricus* LA2	Cow's milk	Submerged fermentation for 24 h at 37 °C (*Enterococcus faecalis*) or 44 °C (*Lactobacillus delbrueckii*)	Peptides with molecular weights less than 5000 Da	Antihypertensive and immune-regulatory
L. acidophilus ATCC 4356 *Lc. lactis* subsp. *lactis* GR5	Sodium Caseinate	Submerged fermentation for 5 h at 30 °C (*Lactococcus lactis*) or 37 °C (*L. acidophilus*) with agitation at 140 rpm	Peptides with molecular weights less than 3000 Da	Immunomodulatory
Aspergillus oryzae	Squid mantles	Solid-state fermentation for 365 days at 25–30 °C	Peptides with molecular weights less than 1450 Da	Antioxidant
B. subtilis 10160	Rapeseed	Solid-state fermentation for 6 days at 32 °C and 85 ± 5% relative humidity	Peptides with molecular weights between 180 and 5500 Da	Antioxidant
Bifidobacterium longum KACC91563	Casein	Submerged fermentation for 24 h	Val-Leu-Pro-Val-Gln	Antioxidant
Lactobacillus casei spp. *pseudoplantarum*	Concentrated soy protein	Submerged fermentation for 36 h at 37 °C	Leu-Ile-Val-Thr-Gln	Antihypertensive
B. subtilis ATCC 6051	Bean	Solid-state fermentation for 96 h at 30 °C and 90% relative humidity	Peptides with molecular weights between 6.2 and 201.2 kDa	Antioxidant Antihypertensive

Produção de Peptideos

Chemical synthesis of peptides

allows the systematic variation of structure with the aim of developing peptides for therapeutic use, and is widely used in structural biology, immunology, protein engineering and biomedical research.

The traditional solution chemical synthesis is a time-consuming process and needs many toxic reagents, which may contribute to significant environmental pollution. Furthermore, it provides poor yields, racemization reactions and peptide by-products.

The synthesis of polypeptides on a solid support is an alternative to traditional methods and dramatically improves yields, decreases the amount of chemicals used and considerably improves the fidelity of peptide chain assembly. Advances in solid-phase peptide synthesis (SPPS) over the past decade have encouraged increased efforts towards the total chemical synthesis of large complex peptides and small proteins. It leads to superior acylation rates, reduces racemization and has higher solubility in the common coupling solvents, e.g. DMF, achieving chain assembly rates of approximately 10–15 amino acid residues per hour.

Sequence analysis of bioactive peptides can be used to predict the potential bioactivity.

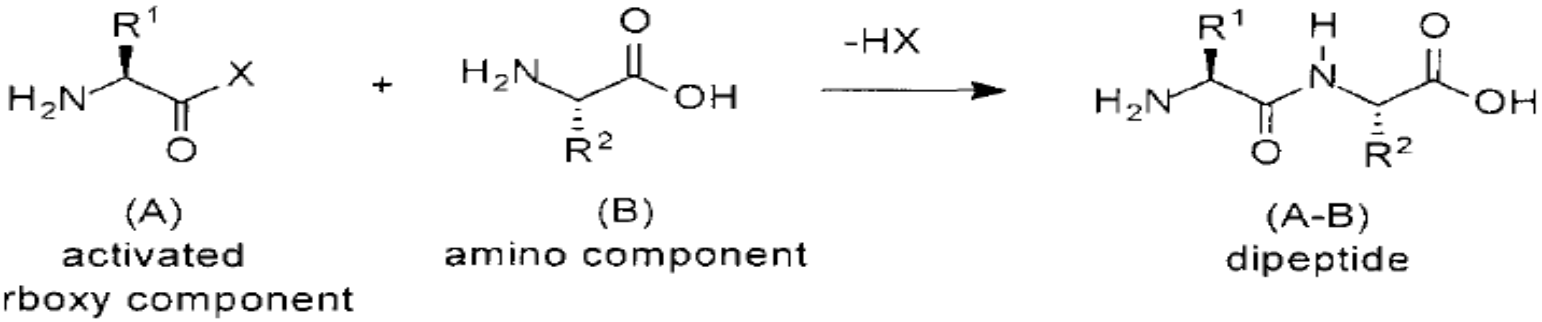
New peptides with improved biological activity as compared to their natural analogues can be designed, and maybe multifunctionality.

Another opportunity: substitution of particular amino acids in the peptide sequence, which can result in modulation or differentiation of peptide function.

Ex1: Replacement of the C-terminal phenylalanine by tryptophan in ovokinin (2–7), resulted in significant improvement of its antihypertensive activity.

Ex2: Replacement of the basic amino acids in particular positions of peptides increases their anticoagulant activity.

Produção de Peptideos



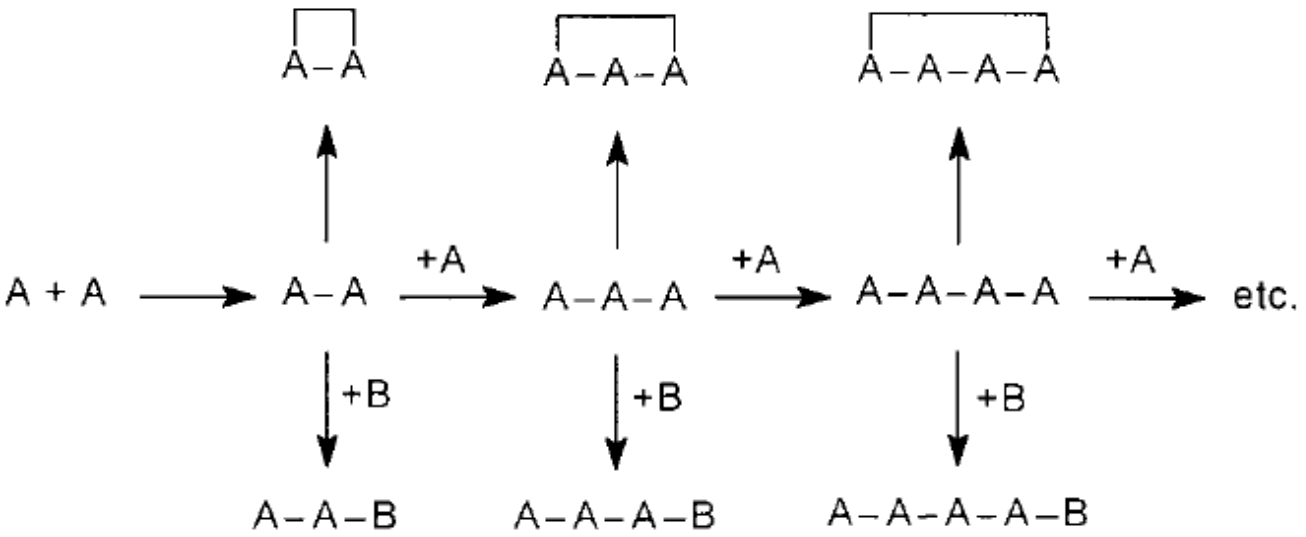


Fig. 4.3 Schematic view of possible side reactions of a peptide synthesis involving an activated N^{α}-unprotected carboxy component (A). Linear and cyclic oligomers are formed besides the desired dipeptide A-B. X=activating group; R^1, R^2=amino acid side chains.

Produção de Peptideos

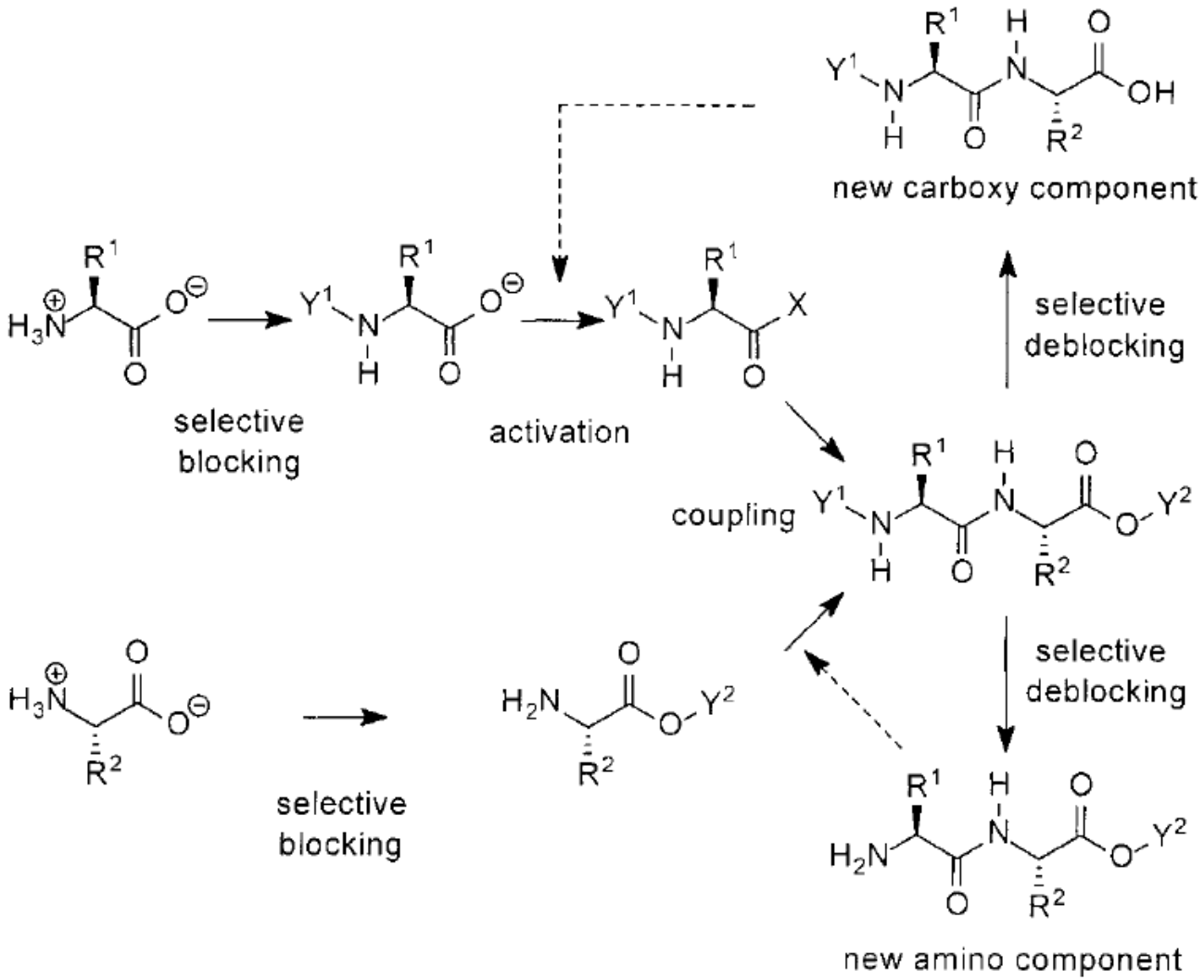
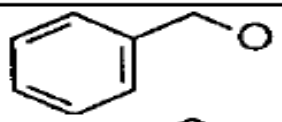
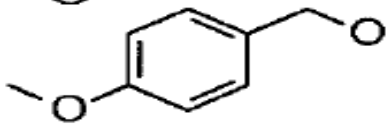
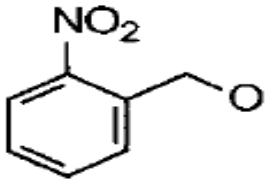
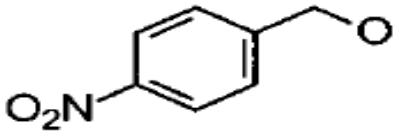
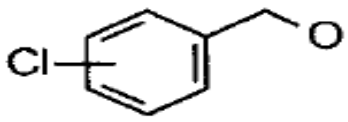


Fig. 4.4 The multi-step process of peptide synthesis. Y^1 = amino-protecting group; Y^2 = carboxy protecting group; R^1, R^2 = amino acid side chains.

Produção de Peptideos

Tab. 4.1 Selected amino-protecting groups of the urethane type Y-O-(C=O)-NH-R^1.

Group	*Symbol*	*Y-O*	*Cleavage conditions*
Benzyloxycarbonyl	Z		H_2/Pd, HBr/AcOH, Na/liq. NH_3
4-Methoxybenzyloxy-carbonyl	Z(OMe)		TFA; H_2/Pd; Na/liq. NH_3
2-Nitrobenzyloxy-carbonyl	Z(2-NO_2)		H_2/Pd (more labile than Z) HBr/AcOH (more stable than Z) photolysis
4-Nitrobenzyloxy-carbonyl	Z(NO_2)		Like Z, but more stable to HBr/HOAc
Chlorobenzyloxy-carbonyl	Z(Cl) Z(3-Cl) Z(2-Cl) Z(2,4-Cl)		Like Z, but more stable to H_2/Pd or HBr/AcOH TFA/CH_2Cl_2

Produção de Peptideos

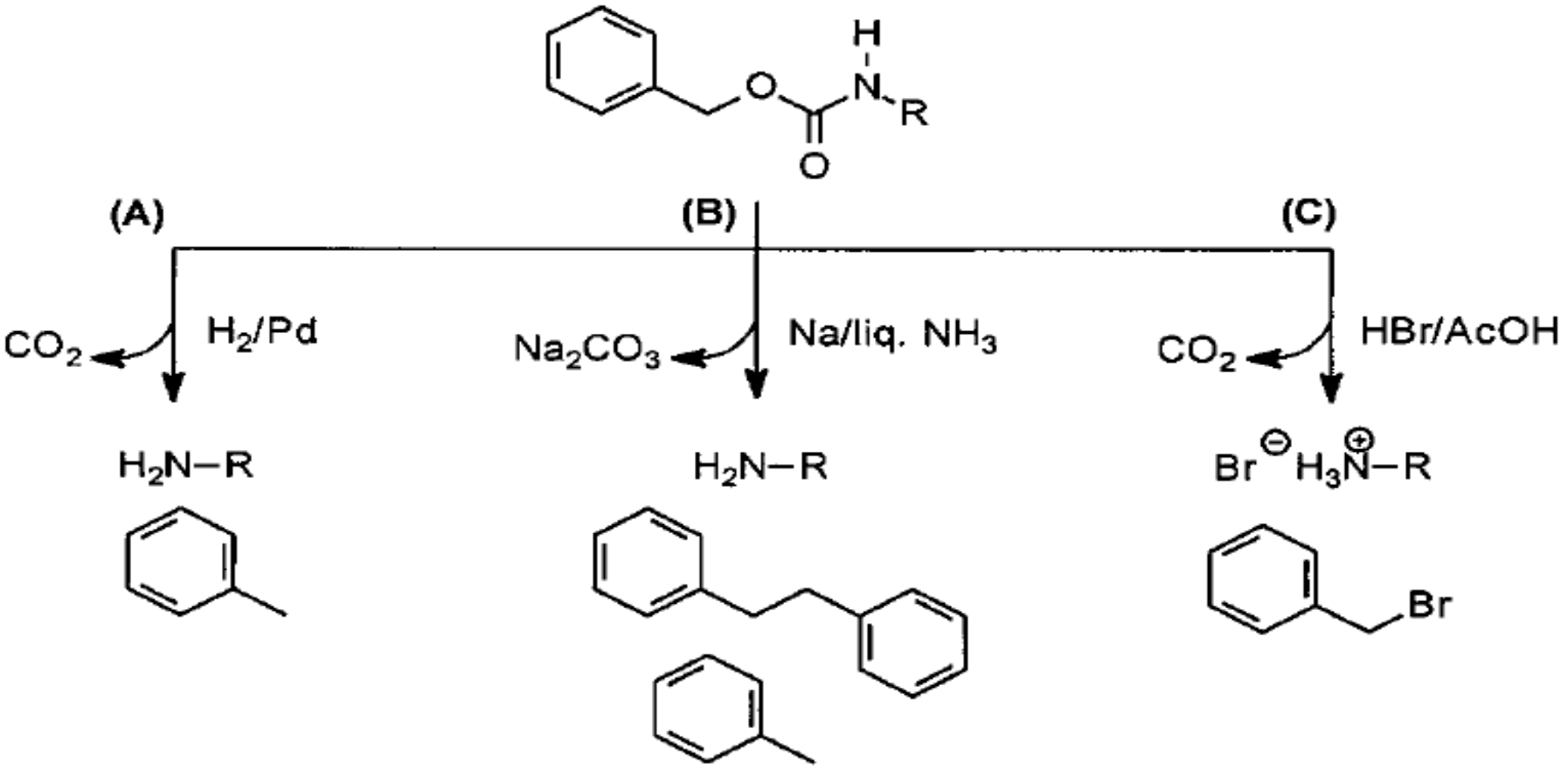


Fig. 4.5 Cleavage conditions of the benzyloxycarbonyl group. H_2N-R=amino acid.

Produção de Peptideos

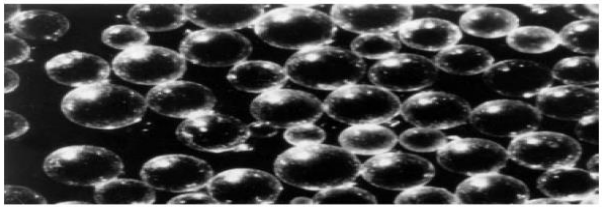


Fig. 4.30 Polystyrene/divinylbenzene cross-linked resin.

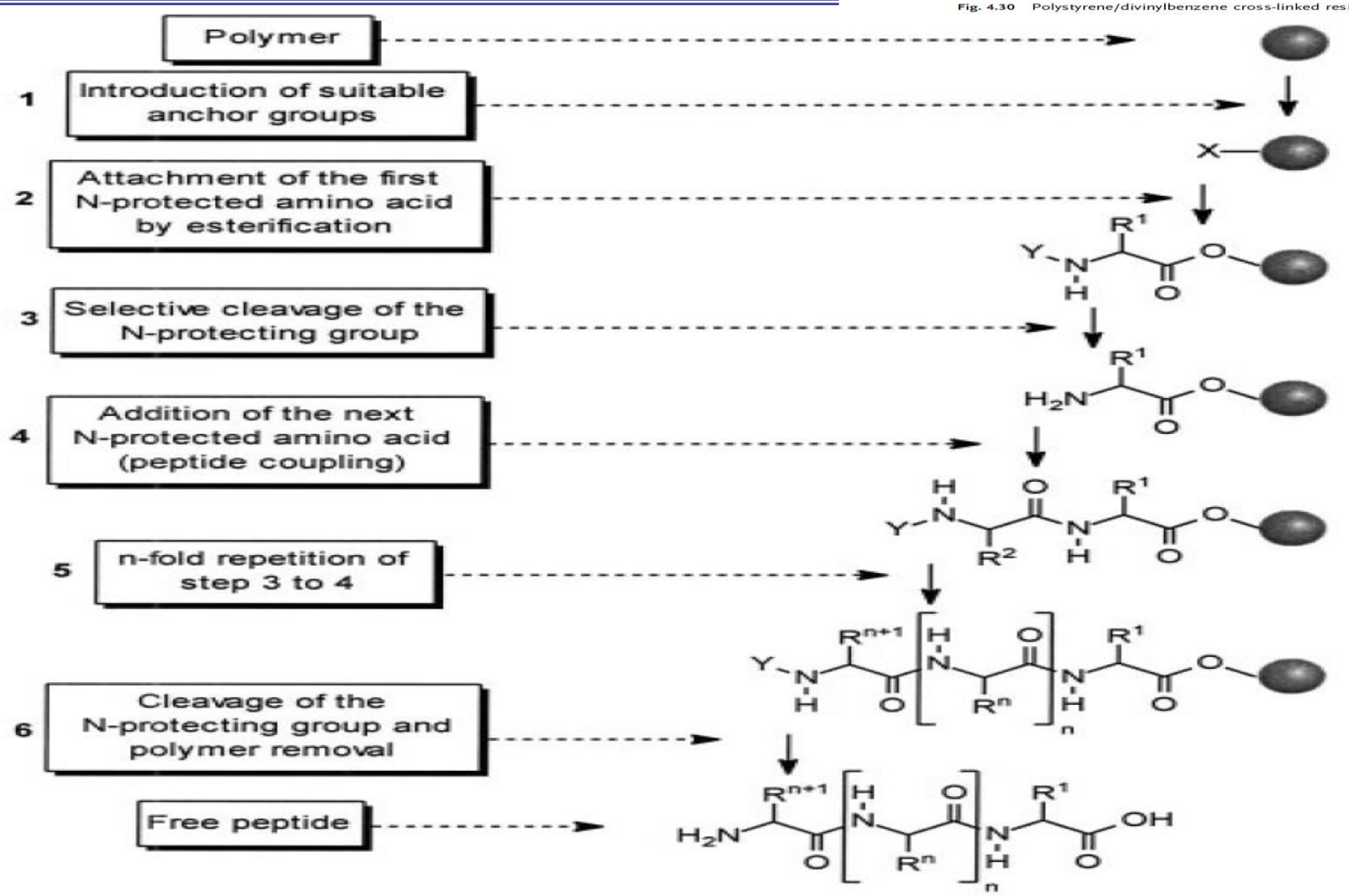


Fig. 4.29 Process of solid-phase peptide synthesis. Y = temporary protecting group; R^1, R^2, R^n, R^{n+1} = amino acid side chains, if necessary, protected with semipermanent protecting group.

Produção de Peptideos

Molecular biology methods

development of synthetic genes for the delivery and expression of bioactive peptides or their precursors in microbial cells.

The manufacture of larger amounts of peptides needed for food supplementation may be possible by using foodgrade dairy fermentation bacteria as peptide production systems. For example two peptides, the 11-residue antimicrobial peptide from bovine lactoferrin (BL-11) (RRWQWRMKKLG) and the 12-residue ACE inhibitory peptide from as1-casein (C-12) (FFVAPFPEVFGK), have been cloned in Streptococcus thermophilus bacteria.

Nucleic acid sequences encoding the peptides were generated by overlapping PCR and were subsequently cloned into a new expression vector. Lactoferricin, an antimicrobial peptide, was successfully multimerized and expressed in Escherichia coli BL21(DE3). About 60 mg of pure peptide with a molecular weight similar to that of the chemically synthesized lactoferricin was obtained from 1 L of E. coli culture.

expression of recombinant protein in the microbial cell followed by the hydrolysis of the fusion protein by microbial proteinaseFor example recombinant lactoferricin B was cleaved successfully to a novel hybrid antimicrobial peptide LFT33 by enterokinase.

A biological expression system using genetic techniques based on a fusion technology would be a more efficient method for the production of bioactive peptides. However, the molecular biology research has led to the availability of amino acid sequence data determined by the cloning and cDNA sequencing of genes. However, in many cases, the proteins themselves have never been isolated and their properties are largely unknown.

Peptidomica

Analytical method of choice for the structural elucidation of peptides and of their post-translational modifications.

1) Pre-purification methods → used to separate peptides from all other molecules (carbohydrates, proteins, lipids, salts. . .).
2) Fractionation techniques → separation of the different peptides based on different physico-chemical properties (size-exclusion chromatography (SEC), liquid chromatography (LC), capillary electrophoresis (CE), gel-free isoelectrofocusing (GF-IEF))
3) Tandem mass spectrometry (matrix assisted laser desorption/ionization-tandem mass spectrometry (MALDI-MS/MS), electrospray-mass spectrometry (ESI-MS)) and bioinformatics are used to identify and quantify all peptides in the complex.

Development prospects

Two main approaches are now reported: the former uses in silico enzymatic digestion and the bioactivity prediction using a combination of sequence biochemical properties and databases of known bioactive peptides; the latter is based on structure-based molecular modeling approach coupled to a docking study (if the 3D structure of the target protein is known) to evaluate the binding affinity between bioactive peptides and their target.

Peptideos Bioativos

The main characteristics of the different analytical methods for the purification and identification of bioactive peptides.

Method of purification/identification	Mechanism	Advantage	Limitation
Reversed phase high pressure liquid chromatography (RP-HPLC)	Based on the hydrophobicity of proteins or peptides that can interact differently to the reversed-phase material of the chromatography column.	Useful method for the isolation of complex peptide mixtures.	Lack of retention of polar molecules. Slow intrapore diffusion times. The presence of unresolved structural microheterogeneity and conformational isomers. Secondary interactions with the stationary phase.
Affinity chromatography	Based on the affinity of bioactive peptides to interact specifically and reversibly with a complementary molecule bound to a solid support immobilized on a column.	The flexibility of using a large number of binding agents, allows for the separation of different types of peptides.	Tone must know the physicochemical properties of the ligands, which limits its use for a complex mixture of unknown peptides.
Ion-exchange chromatography (IEC)	Based on the ability of charged bioactive peptides to interact with a solid support bearing the opposite charge.	Appropriate method for the separation of highly cationic or anionic peptides.	Low selectivity and requires complementary steps for the separation of the fractions.
Isoelectric focusing (IEF)	Based on the separation of protein/peptide solutions according to their isoelectric points (pI). A focusing cell containing a mixture of proteins/peptides and a carrier ampholyte is subjected to an electric potential, causing the migration of the proteins/peptides to a position in an established pH gradient equivalent to their respective pI.	The method allows one to fractionate a complex mixture of peptides according to their pI.	Loss of highly hydrophobic proteins in the sample preparation and precipitation of neutral proteins at their pI, which can result in overlapping between different fractions.
Size exclusion chromatography (SEC)	Based on the fractionation of bioactive peptides according to the retention time of the molecules in the stationary phases particles with a carefully controlled pore size, in which the molecules are separated from each other according to their molecular size.	The elution conditions are considered mild, allowing the characterization of the protein with minimal impact on the conformational structure and the local environment.	Long columns are required for complex peptide mixtures, which can be obtained by joining multiple columns in a series. This strategy is necessary to improve the separation resolution.

Peptideos Bioativos

Ultra high pressure liquid chromatography (UHPLC)	Based on separation of the molecules using experimental columns packed with very small particles of a non-porous material, carrying out the analyses at very high pressures.	Increased throughput, resolution and sensitivity in separation of complex protein mixtures.	The heat dissipated from the use of small particles at ultra-high pressures may increase chromatographic band broadening and compromise efficiency of the column.
Hydrophilic interaction liquid chromatography (HILIC)	Based on the polarity and hydrophilicity of bioactive peptides separated using polar chromatographic surfaces (stationary phase) and a highly organic mobile phase (>70% solvent) also containing a small percentage of aqueous solvent/buffer or other polar solvent.	The method shows great potential for the separation of short peptide sequences (<5 amino acids) and improves the identification using mass spectrometry.	Compared to RP-HPLC, the method shows limited flexibility and applicability, problems with sample solubility and the retention mechanisms are poorly understood.
Electrospray ionization mass spectrometry (ESI/MS)	Based on the transformation of an aqueous solution with uniform electrical density to gas-phase ions, by passing a high voltage through a thin capillary. The gas-phase is transferred into a mass analyzer and separated according to the mass-to-charge (m/z) ratio.	Production of singly and multiply charged ions, allowing for an accurate measurement of the molecular weight of the peptides.	The efficiency of identification is directly related to the chromatographic method used for the prior separation of the bioactive peptides before injection into the mass spectrometer. Therefore, a combination of different separation techniques is necessary for accurate identification.
Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF/MS)	Based on co-crystallization of the analytes when they are mixed with a matrix solution on a target plate. The co-crystal is subjected to the action of pulsed laser, causing the accumulation of high-density energy which results in vaporization of the analyte and matrix molecule. MALDI is usually connected to TOF mass spectrometer which measures the flight time of ions to the ion detector, and provides the m/z ratio mapping results.	The method has no theoretical upper limit to the m/z ratio, allowing for the analysis of complex samples with a wide range of molecular weights.	

Peptideos Bioativos

The hydrolysates firstwere subjected to ultrafiltration through a 1-kDa membrane, and peptides with molecular weights lower than this cutoff were collected. Purification consisted of sequential steps of isolation by fast protein liquid chromatography (FPLC) (AKTA, Amersham Bioscience Co., Uppsala, Sweden) using a HiPrep 16/10 high flow ionic exchange column (16 × 100 mm, Amersham Biosciences, Piscataway, NJ, USA) and a GE Healthcare Superdex™ Peptide 10/300 GL gel filtration column (10 × 300 mm).

In a complex mixture of peptides, common problems are the separation of small and big peptides or peptides with different physicochemical properties, which makes their subsequent identification difficult. These problems can be solved by a combination of different separation techniques before injection into the mass spectrometer. A practical example is the separation of peptides containing hydrophobic amino acids and peptides composed of only hydrophilic amino acids. In this case, a combination of RP-HPLC with HILIC can be used for an efficient separation of the peptides with hydrophobic and hydrophilic characteristics, respectively.

Separação e Purificação

- Ultrafiltração
- Nanofiltração
- Eletrodiálise

Choice of membrane, implementation mode and operating condition was rather empirical (non specific to the peptide of interest and the starting hydrolysate properties).

This method couples (i) SEC chromatogram of the hydrolysate and targeted peptide molecular weight to mass balance equations and membrane calibration.

Fractionation processes have been carried out in taking account hydrophobicity property of peptides; ion-pairing assisted extraction in water/organic solvent biphasic systems. Ion pair formation in an aqueous phase, thanks to the addition of alkyl sulfonates or sodium dodecyl sulfate agents, allowed to selectively extracting these bioactive peptides in organic phases consisting of dichloromethane or octan-1-ol. Such extraction system was intensified using an ultrafiltration membrane

Other authors have worked on the combination of a membrane contactor and an organic interface consisting of octan-1-ol for the extraction of opioid peptides. Another way to intensify a solvent extraction system is to implement it in centrifugal partition chromatography (CPC).

They have isolated antibacterial peptides in a foaming-draining process in exploiting differences in surfactant properties of the peptides present in the hydrolysate, related to differences in hydrophilicity, hydrophobicity and molecular size.

Fatores de Crescimento

São proteínas reguladoras capazes de mediar vias sinalizadoras inter e intracelulares, tendo papel importante na cicatrização de ferimentos, induzindo a formação de colageno, elastina e glicosaminoglicanas, além de angiogenese → melhora a aparencia de pele fotoenvelhecida, diminuendo rugas e irregulridades. Normalmente, hão há efeitos colaterais, mas pode ocorrer reações alergicas em pacientes hipersensiveis.

Epidermal Growth Factor (EGF)

Normally present in many biological secretions, including plasma, sweat, urine, and saliva. After its binding to the epidermal growth factor receptor (EGFR), it stimulates epidermal growth and differentiation. It has been reported to be useful as a re-epithelization agent in the treatment of burns and excision wounds.

Transforming Growth Factor (TGF)

TGF stimulates physiological skin growth, cellular growth, and skin repair. It promotes re-epithelization and mediates fibrosis. TGF is involved in tissue repair and angiogenesis. It may increase the risk for hypertrophic scarring, because of its function in activating fibroblasts.

Peptideos Bioativos

GROWTH FACTORS AND CYTOKINES

Growth factors are produced and secreted by many cell types of the skin, including fibroblasts, keratinocytes, and melanocytes. Included within these secreted growth factors are those that regulate the immune system, also known as cytokines.

One of the concerns with growth factors, as with other peptides, is their size and ability to penetrate the epidermis. Growth factors and cytokines are very large molecules, usually more than 15 kDa, and are hydrophilic. However, it is proposed that absorption occurs by way of hair follicles, sweat glands, and compromised skin. Once absorbed into the epidermis, communication is able to occur between epidermal cells and cells of the dermal layers. Growth factors are also very unstable outside of their physiologically active environment unless stored at –20C, which is clearly not practical for the use of cosmetic application. One study, however, showed that growth factors and cytokines remained stable for more than 24 months.

Growth factors regulate cell growth and thus have a potential for carcinogenic transformation of cells. EX. vascular endothelial growth factor (VEGF). Receptors for VEGF are present on some types of melanoma cells. FDA investigation of a growth factor product determined that very large concentrations of growth factors, much higher than the levels found in topical cosmeceutical products, are required for this potential. Furthermore, because of the large size, minimal amounts of growth factors enter the skin. Thus, topical application of growth factors is unlikely to have any effect on cancer growth.

One source for growth factors, cytokines, and matrix proteins is neonatal dermal fibroblasts, which secrete more than 110 of these combined products

Table 2
Growth factors and cytokines found in cosmeceutical products

Growth factors	Fibroblast growth factor	Activates fibroblasts, angiogenic, induces collagen synthesis
	Heparin binding-epidermal growth factor	Keratinocyte and fibroblast mitogen
	Hepatocyte growth factor	Tissue regeneration and wound healing
	Insulin-like growth factor	Activates fibroblasts and endothelial cells
	Placenta growth factor	Activates fibroblasts, promotes endothelial growth
	Platelet-derived growth factor	Induces fibroblast migration, fibroblast mitogen, matrix production
	Transforming growth factor-β1	Induces keratinocyte, fibroblast, and macrophage migration, and angiogenesis Initiates collagen and fibronectin synthesis; modulates degradation of matrix proteins
	Transforming growth factor-β2	Induces keratinocyte, fibroblast, and macrophage migration; initiates collagen and fibronectin synthesis
	Transforming growth factor-β3	Antiscarring
	Vascular endothelial growth factor	Inhibits collagen and hyaluronic acid degradation
Cytokines	IL-1α and -1β	Activates growth factor expression in macrophages, keratinocytes, and fibroblasts
	IL-1ra	Anti-inflammatory
	IL-10	Anti-inflammatory
	IL-13	Anti-inflammatory
	Tumor necrosis factor-α	Activates growth factor expression in macrophages, keratinocytes, and fibroblasts

Receptores

Quadro 23. Receptores específicos presentes na pele

Célula	*Receptores presentes*
Queratinócitos	α-MSH, ACTH, epinefrina, CRH, vitamina D, cortisol, RAR, eicosanoides
Folículo capilar	ACTH, α-MSH, b-endorfinas, CRH
Nervos cutâneos	CRH
Sebócitos	Andrógenos, estrógenos, vitamina D, cortisol, RAR
Melanócitos	IGF-1, ACTH, α-MSH, CRH
Fibroblastos	IGF-1 e 2, IGFBP-3, ACTH, α-MSH, b-endorfina

Fonte: Y. Kariya *et al.*, "Sex steroid hormone receptors in human skin appendage and its neoplasms". Em *Endocrine Journal*, 52 (3), 2005.